Overall survival for neuroblastoma in South Africa between 2000 and 2014

Van Heerden, Jaques[1]; Hendricks, Marc[2]; Geel, Jennifer[3]; Sartorius, Benn[4,5]; Hadley, GP[6]; Du Plessis, Jan[7]; Büchner, Ané[8]; Naidu, Gita[9]; Van Emmenes, Barry[10]; Van Zyl, Anel[1]; Kruger, Mariana[1]

Affiliations

[1] Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg Hospital, Cape Town, South Africa*

[2] Haematology Oncology Service, Red Cross War Memorial Children's Hospital, Department of Paediatrics and Child Health, Faculty of Health Sciences, University of Cape Town, South Africa

[3] Faculty of Health Sciences, Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, University of the Witwatersrand, Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa

[4] Discipline of Public Health Medicine, School of Nursing and Public Health, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

[5] Department of Health and Medical Sciences, University of Washington, Seattle, Unites States of America

[6] Department of Paediatric Surgery, Faculty of Health Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa

[7] Department of Paediatrics, Faculty of Health Sciences, University of the Free State, Division of Paediatric Haematology and Oncology, Universitas Hospital, Bloemfontein, South Africa

[8] Paediatric Haematology and Oncology, Department of Paediatrics, University of Pretoria, Steve Biko Academic Hospital, Pretoria, South Africa

[9] Faculty of Health Sciences, Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, University of the Witwatersrand, Chris Hani Baragwanath Academic Hospital, Johannesburg, South Africa

[10] Division of Paediatric Haematology and Oncology, Department of Paediatrics, Frere Hospital, East London, South Africa

* The primary author is a PhD student at Stellenbosch University, South Africa, but a permanent staff member of the Antwerp University Hospital, Antwerp, Belgium.

Correspondence to: Dr Jaques van Heerden, Department of Paediatric Haematology and Oncology, University Hospital of Antwerp, Wilrijkstreet 10, Edegem 2650, Belgium. E-mail: Jaques.vanheerden@uza.be

Contributors' statement

Jaques van Heerden conceptualised and designed the study, collected the data and performed the data analysis.
Mariana Kruger assisted with concept development and design of the study, supervised the data analysis, and critically reviewed and revised the manuscript.
Benn Sartorius performed the statistical analysis and critically reviewed the manuscript.
All the other authors collected data in their respective paediatric oncology units and critically reviewed and contributed toward revision of the manuscript.

Word count

Abstract: 233

Main text: 4527

Running title: OS for neuroblastoma in SAfrica between 2000 and 2014

Features

Tables: 3

Supplementary tables: 8

Supplementary figure: 1

Graphs: 3

Appendices: 2

Abbreviations

CI	Confidence interval
CR	Complete response
CT	Computed tomography
COG	Children's Oncology Group
FDG/PET	^{129}Fluorodeoxyglucose positron emission tomography
FNA	Fine-needle aspiration
HFA	Height for age

HIC	High-income countries
HIV	Human immunodeficiency virus
HR	High-risk
INPC	International Neuroblastoma Pathology Classification
IDRF	Image-defined risk factors
INRC	International Neuroblastoma Response Criteria
INRG	International Neuroblastoma Risk Group
INSS	International Neuroblastoma Staging System
IR	Intermediate-risk
LDH	Lactate hydrogenase
LR	Low-risk
MIC	Middle-income country
MRI	Magnetic resonance imaging
NB	Neuroblastoma
OMAS	Opsoclonus myoclonus ataxia syndrome
OS	Overall survival
PD	Progressive disease
POU	Paediatric oncology units
SACCSG	South African Children's Cancer Study Group
SACTR	South African Children's Tumour Registry
TB	Tuberculosis
US	Ultrasonography
VIP	Vasoactive intestinal peptide
VLR	Very-low-risk
WFA	Weight for age

Keywords

South Africa, neuroblastoma, outcomes, overall survival

Abstract

Background

Outcome data for neuroblastoma in sub-Saharan Africa are minimal while poor outcome is reported in low- and middle-income countries. A multi-institutional retrospective study across South Africa was undertaken to determine outcome.

Methods

Patients treated between January 2000 and December 2014 in nine South African paediatric oncology units were included. Kaplan-Meier curves and Cox regression models were employed to determine two-year survival rates and to identify prognostic factors.

Results

Data from 390 patients were analysed. The median age was 39.9 months (range 0-201 months). The majority presented with Stage 4 disease (70%). The main chemotherapy regimens were OPEC/OJEC (44.8%), St Jude NB84 protocol (28.96%) and Rapid COJEC (22.17%). Only 44.4% had surgery across all risk groups while only 16.5% of high-risk patients received radiotherapy. The two-year overall survival (OS) for the whole cohort was 37.6%: 94.1%, 81.6% and 66.7% respectively for the very-low-risk, low-risk and intermediate-risk groups and 27.6% for the high-risk group ($p < 0.001$, 95% CI). The median survival time for the whole group was 13 months (mean 41.9 months, range 0.1-209 months). MYCN-non-amplified patients had a superior two-year OS of 51.3% in comparison to MYCN-amplified patients at 37.3% ($p = 0.002$, 95% CI).

Conclusions

Limited disease had an OS comparable to high-income countries, but advanced disease had a poor OS. South Africa should focus on early diagnosis and implementation of a national protocol with equitable access to treatment.

1. Introduction

Neuroblastoma (NB) is a malignancy of the sympathetic nervous system during early childhood with a worsening prognosis with increasing age.[1] NB presents various management challenges in high-income countries (HICs) and more so in resource-limited settings.[2] The varied pathophysiological, biological and genetic characteristics contribute to differing treatment approaches for low-risk (LR) disease (local disease, favourable histology, no raised tumour markers, MYCN non-amplification and no adverse genetics), with relatively little intervention required beyond surgery. Intermediate-risk (IR) disease has LR features combined with a single genetic aberration such as 11q and diploidy or undifferentiated pathology.[3] IR disease is treated with chemotherapy and surgery with radiotherapy indicated for limited indications.[3] Multimodal therapy (chemotherapy, surgery, radiotherapy, myelo-ablative stem cell transplantation, immunotherapy and maturation therapy) is vital for improved outcomes in high-risk (HR) disease (metastatic disease, unfavourable histology, raised tumour markers, MYCN amplification and adverse genetics).[3] Despite the use of high-intensity treatment for HR disease, the outcomes for LR and HR disease are vastly dissimilar.[3,4] Epigenetics is emerging as a factor influencing outcomes.[5]

South Africa is an upper-middle-income country with a heterogeneous healthcare system, consisting of public and private care facilities.[6,7] Most children with malignancies are treated in the public sector where the level of care varies according to provincial resources and institutional preference.[6] The treatment of NB in South Africa, like elsewhere in sub-Saharan Africa, is further complicated by several factors, including an HIV epidemic and sociocultural differences in disease interpretation, contributing to late presentation, advanced disease and increased abandonment

rates.[8,9] This retrospective study aimed to review NB management and outcomes in South Africa with the overarching aim to develop a standardised national NB treatment protocol.

2. Methods

2.1. Patient population

There were 564 newly diagnosed registered cases of NB between January 2000 and December 2014 from nine dedicated paediatric oncology units (POUs) and the private health care sector in South Africa (see flow diagram) in the South African Children's Cancer Study Group (SACCSG) South African Children's Tumour Registry (SACTR).[10] Data from 174 (30.9%) patients' files were not available. Access to files from most private hospital facilities was not available and incomplete or loss of files from archives had to be omitted from data analysis.

2.2. Setting

The study was conducted in nine POUs, servicing both rural and urban populations from varying socio-economic backgrounds.[11] Only four POUs had access to stem cell transplant services and seven had nuclear imaging services. There is still a disparity in resource allocation between different POUs in the nine provinces in SA.[11,12] This impacted on both the available human and physical resources availability. During the study period autologous stem cell transplantation and cis-retinoic acid were largely limited to patients with private health care insurance. Immunotherapy with anti-GD2 was and is not available in South Africa.

2.3. Data collection

Age was defined in months. End point was defined as two-year overall survival (OS). Treatment failure was calculated from date of diagnosis until date of disease progression, or relapse or death due to NB. Refractory disease was defined as disease in which a complete response (CR) with initial induction was not obtained. Early and late relapse were respectively defined as relapse within or more than six months after completion of therapy. Treatment abandonment was defined as failure to initiate or complete treatment with a curative intent, except when the treating physician elected to opt for palliative management. If a patient did not attend follow-up appointments and if contact with family or caregivers was unsuccessful for a duration of two years, the patient was considered lost to follow-up.

2.4. Staging and imaging

Investigations included ultrasonography (US), computed tomography (CT) or magnetic resonance imaging (MRI) of the neck, chest and abdomen as well as bone marrow aspiration and trephine biopsy. Patients were clinically and radiologically staged according to the International Neuroblastoma Staging System (INSS) or image-defined risk factors (IDRFs).[3] The INSS included unfavourable histology or favourable histology and MYCN amplification as part of the risk classification. Depending on institutional availability, staging and skeletal screening were done by ^{123}I-MIBG scan and/or bone scan. In ^{123}I-MIBG scan-negative patients, when available, staging with ^{129}fluorodeoxyglucose positron emission tomography/CT (FDG PET/CT) was performed. Primary tumour and metastatic response evaluations were performed according to institutional availability based on the International Neuroblastoma Response Criteria (INRC).[13,14,15] The treatment response evaluations were done

according to the most recent version of the criteria pertaining to the specific investigations used to determine the response.

2.5. Risk classification

Risk classification was based on the Children's Oncology Group (COG) classification system (Appendix A) and the International Neuroblastoma Risk Group (INRG) Consensus Pre-treatment Classification Scheme (Appendix B).[3] Lactate hydrogenase (LDH) and ferritin were used as nonspecific tumour markers as per the SIOP-PODC adapted risk stratification and treatment guidelines[2] that specify that a level of > 750 UI/L for LDH and > 120 ng/ml for ferritin is indicative of a poor prognosis. INRG was used for statistical purposes.

2.6. Treatment

During the study period, different treatment protocols were used according to either institutional preference or disease severity. Regimens consisted of a surgical approach in very-low-risk (VLR), LR, intermediate-risk (IR) and HR disease, and chemotherapy in IR and HR disease (employed as neo-adjuvant or adjuvant chemotherapy) and with progression in LR disease. Conventional radiotherapy was included according to protocols in IR and HR disease. Surgical intervention was dictated by the resectability of the tumour, response to chemotherapy and surgical expertise in the local institution.

2.6.1. Chemotherapy regimens

Patients were, regardless of risk group, treated with a spectrum of regimens ranging from observation to platin- and etoposide-based regimens, which included

OPEC/OJEC (carboplatin, cisplatin, etoposide, cyclophosphamide and vincristine),[16] the St Jude NB84 protocol (cisplatin, etoposide, doxorubicin and cyclophosphamide)[17] and Rapid COJEC (carboplatin, cisplatin, etoposide, cyclophosphamide and vincristine).[16] CCG-321-P2 (cyclophosphamide, doxorubicin, cisplatin and etoposide)[18] and VACEpi (vincristine, actinomycin, cyclophosphamide and epirubicin) were used to a lesser degree. CADO (cyclophosphamide, adriamycin and vincristine)[2,19] or single-agent oral cyclophosphamide was used as palliative chemotherapy options in certain centres.

2.6.2. Surgery

Surgery was performed for diagnostic reasons or as primary treatment in patients with LR and IR disease with curative intent with adequate neo-adjuvant treatment. Other indications were palliative or emergency purposes. Emergencies included spinal cord compression or bowel, airway and urinary tract obstructive symptoms. In patients with HR disease, surgery was performed based on upfront resectability of the primary tumour or after demonstrable cytoreduction with neo-adjuvant chemotherapy.

2.6.3. Radiotherapy

Radiotherapy was given according to the indications and doses of either the following protocols: St Jude NB84,[17] CCG-321-P2[18] or International Society of Paediatric Oncology European Neuroblastoma Group (SIOPEN) OPEC/OJEC or Rapid COJEC.[2,16] Only six institutions offered maturation therapy of cis-retinoic acid after completion of primary treatment per the abovementioned protocols.[17]

2.6.4. Autologous stem cell transplant

Autologous stem cell transplantation was available to four POUs, mainly for patients with private health care insurance.

2.7. Statistical analysis

Data were analysed using Stata 15.0. Differences in medians and means were assessed using the Mann-Whitney U test or Student's t-test. In cohorts of less than five observations, the Fisher's exact test was applied. The Pearson chi-square test (χ^2) was used to assess the categorical association among covariates. OS as well as two-year survival, with associated 95% confidence intervals, was calculated and described using Kaplan-Meier curves. Differences between bi- and multivariate survival curves by group were assessed using a log rank test. Multiple Cox regression modelling to assess statistical significance of various prognostic factors was employed. The proportional hazards assumption was also confirmed for the final multivariable model. A p-value of less than 0.05 was considered significant for all calculations.

3. Results

3.1. Presentation and comorbid diseases (see Supplemental Table S1)

The final analysis included 390 patient records (see Supplemental Figure 1), after 174 patients' files (30.9%) had been excluded as data were lacking or the diagnosis had not been confirmed. There was a male predominance with a male to female ratio of 1.08:1, and patients had been most frequently diagnosed in the 18-60-months category (50.7%). The median age was 39.9 months (range 0-16.7 years, mean 30.5 months). The diagnosis had been established by core biopsy in 208 patients (53.3%), fine-needle aspiration (FNA) in 62 patients (15.9%) and bone marrow biopsy in 120

patients (24.4%) with bone marrow involvement, with bone marrow infiltration by NB in association with increased urine catecholamine levels or high index of radiological suspicion. Chemotherapy data on 10 patients (2.7%) were insufficient for analysis, and these records were excluded. In 168 patients (47.5%), chemotherapy had to be interrupted or adjusted due to comorbid disease, advanced disease, severe toxicity or absconding.

The most common presentation was a clinically palpable mass (43.3%) followed by metastatic skull lesions (18.4%), bone pain (17.9%) and loss of weight or anorexia (13.3%). A unilateral adrenal mass (75.1%) was the most frequently detected primary mass, followed by a thoracic mass (11.2%). A primary tumour could not be identified in eight (2.1%) patients. Only 4.6% of the patients presented with spinal symptoms. Bone metastases were present in 47.4%, and the bone marrow was infiltrated in 44.6% of patients.

Comorbid disease was present in 21 patients with 11 (2.8%) being HIV positive: nine were on antiretroviral therapy at diagnosis. Ten patients had tuberculosis (TB) (2.5%), with five (1.25%) on TB treatment and five (1.25%) newly diagnosed. Opsoclonus myoclonus ataxia syndrome (OMAS) and vasoactive intestinal peptide (VIP) were present in 0.51% (n = 2) and 0.26% (n = 1) of patients, respectively.

3.2. Diagnostics *(see Supplemental Table S2)*

Diagnostic investigations included chest X-rays (163, 23.1%), plain-film skeletal surveys (45, 11.5%) and skull X-rays (41, 10.5%). Abdominal ultrasounds were done in 205 children (52.6%). CT/MRI scans of chest and abdomen were done in 299 (76.7%) patients, and 136 (34.6%) of CT/MRI scans included screening for skeletal metastasis. Nuclear studies included 157 (40.3%) scintigraphy investigations and 143

(41.8%) ^{123}I-MIBG studies. Three hundred and twenty-one (82.3%) staging bone marrow aspirates were done, of which 204 (63.6%) identified infiltration by NB cells. Only 163 (41.8%) had complete staging which included biopsy proven disease, a ^{123}I-MIBG scan and bone marrow aspirate.

3.3. Age, sex, stage and risk classifications (see Supplemental Table S3)

Age at diagnosis was prognostic with a better two-year OS of 58.9% for age less than 18 months compared to older than 18 months (25.8%; $p < 0.049$, 95% CI) (see Fig. 1). Sex ($p = 0.3$, 95% CI) was not significant in either univariate or multivariate analysis (see tables 1 and 2).

According to the INSS, 273 (70%) patients had Stage 4 disease and 14 (3.6%) had Stage 4S disease. Only 17 (4.3%) patients were classified as VLR, 26 (6.6%) as LR, 30 (7.7%) as IR and 275 (75.6%) as HR. The most common metastatic site was bone (n = 185, 47.4%) followed by bone marrow at 44.6% (n = 174). Lung metastases were present in 3.8% (n = 15). The two-year OS for the cohort was 37.6%, with stage being statistically significant for OS ($p < 0.01$, 95% CI). According to the INSS, patients with Stage 1 and Stage 2A disease had a two-year OS of 100%, those with Stage 2B disease an OS of 74%, those with Stage 3 disease an OS of 63.4%, those with Stage 4 disease an OS of 23.8% and those with Stage 4S disease an OS of 64.4% ($p < 0.001$, 95% CI) (see Fig. 2). According to risk classification, patients with VLR disease had a two-year OS of 94.1%, those with LR disease an OS of 81.6%, those with IR disease an OS of 66.7% and those with HR disease an OS of 27.6% ($p < 0.001$, 95% CI) (see Fig. 3).

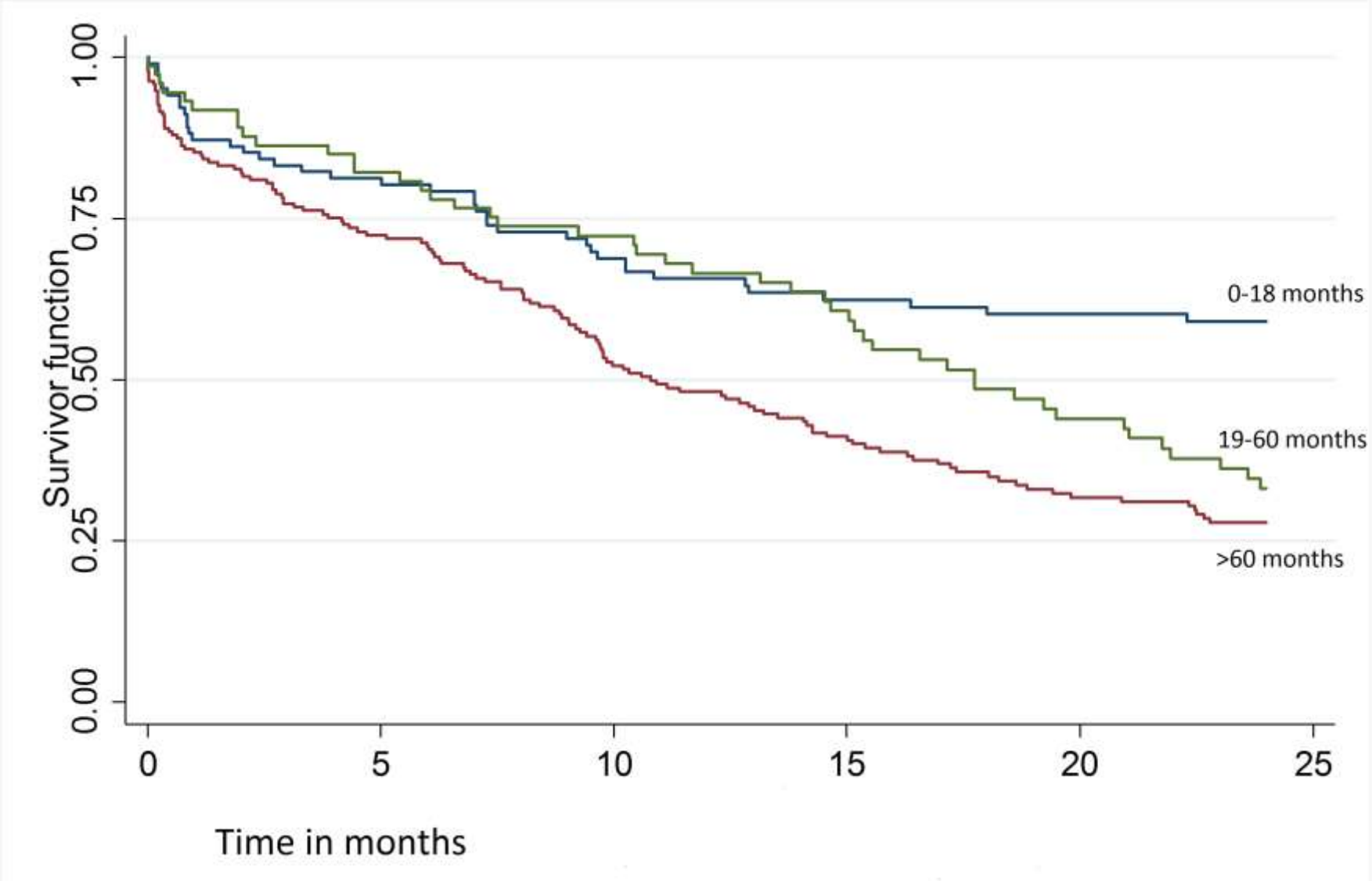

FIGURE 1. Kaplan–Meier curves of the two-year OS for age (*P* = 0.049)

TABLE 1: Prognostic factors and overall outcomes (univariate analysis)				
Prognostic factor	**n**	**Percentage**	**2-year OS**	**p-value CI = 95%**
Age at diagnosis (months)				
Range (months)	0–201			
Average (months)	30.5			
Median (months)	39.9			
0–18 months	123	31.5%	58.9%	< 0.049
18–60 months	191	49%	25.8%	
> 60 months	76	19.5%	33%	
LDH				
< 750 U/L	137	42.9%	54.8%	< 0.0001
> 750 U/L	182	57.1%	25.2%	
Ferritin				
< 120 ng/dl	75	31.9%	66.3%	0.0004
> 120 ng/dl	159	69.1%	25.6%	
MYCN				
Total	145			
Not amplified	64	44%	51.3%	0.002
Amplified	78	54%	37.3%	
Extra copies	3	2%	0%	
U-catecholamine levels				
Total	271			
Normal	50	18.4%	63.4%	< 0.049
Raised	221	81.6%	34.9%	
Pathology				
Total	390			
Favourable histology	80	20.5%	66.6%	0.002
Unfavourable histology	128	32.8%	32.8%	
FNA	62	15.9%		
Bone marrow cytology	120	30.8%	24.4%	
INSS				
1	16	4%	100%	< 0.0001
2A	8	2.1%	100%	
2B	10	2.6%	74%	
3	68	17.4%	63.8%	
4	273	70%	23.8%	
4S	14	3.6%	64.6%	
Risk classification				
VLR	17	4.3%	94.1%	< 0.0001
LR	26	6.6%	81.6%	
IR	30	7.7%	66.7%	
HR	295	75.6%	27.6%	
LDH – lactate hydrogenase; FNA – fine-needle aspiration; INSS – International Neuroblastoma Staging System; VLR – very low risk; LR – low risk; IR – intermediate risk; HR – high risk				

TABLE 2: Multivariate analysis							
Covariate	**Coefficient**	**Standard Error**	**Wald**	**p-value**	**Exp**	**95% CI**	
						Min	**Max**
Age	0.1490	0.07038	4.4817	0.0343	1.1607	1.0111	1.3323
Race	0.09036	0.1066	0.7183	0.3967	1.0946	0.8882	1.3489
Sex	-0.1366	0.1361	1.0084	0.3153	0.8723	0.6681	1.1389
MYCN	0.1622	0.06318	6.5943	0.0102	1.1761	1.0392	1.3312
LDH	0.4862	0.09801	24.6057	< 0.0001	1.6261	1.3419	1.9704
Ferritin	0.2230	0.09526	5.4786	0.0193	1.2498	1.0369	1.5063
INPC	0.09132	0.1025	0.7941	0.3729	1.0956	0.8962	1.3393
INSS	0.8876	0.1959	20.5212	< 0.0001	2.4293	1.6546	3.5667
Risk classification	0.5555	0.1106	25.2176	< 0.0001	1.7428	1.4031	2.1648
LDH – lactate hydrogenase; INPC – International Neuroblastoma Pathology Classification; INSS – International Neuroblastoma Staging System							

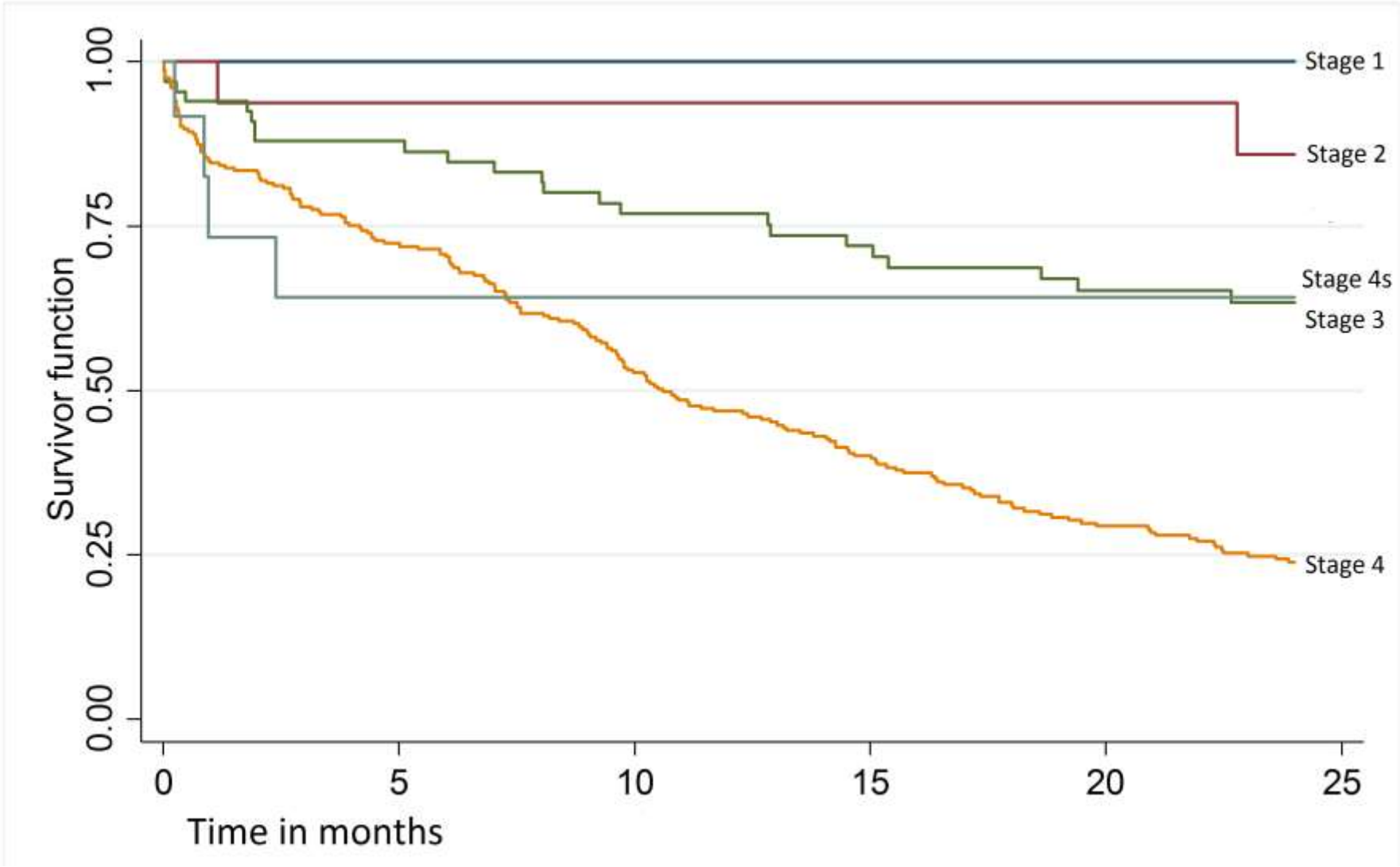

FIGURE 2. Kaplan–Meier curves of the two-year OS for INSS ($P < 0.001$)

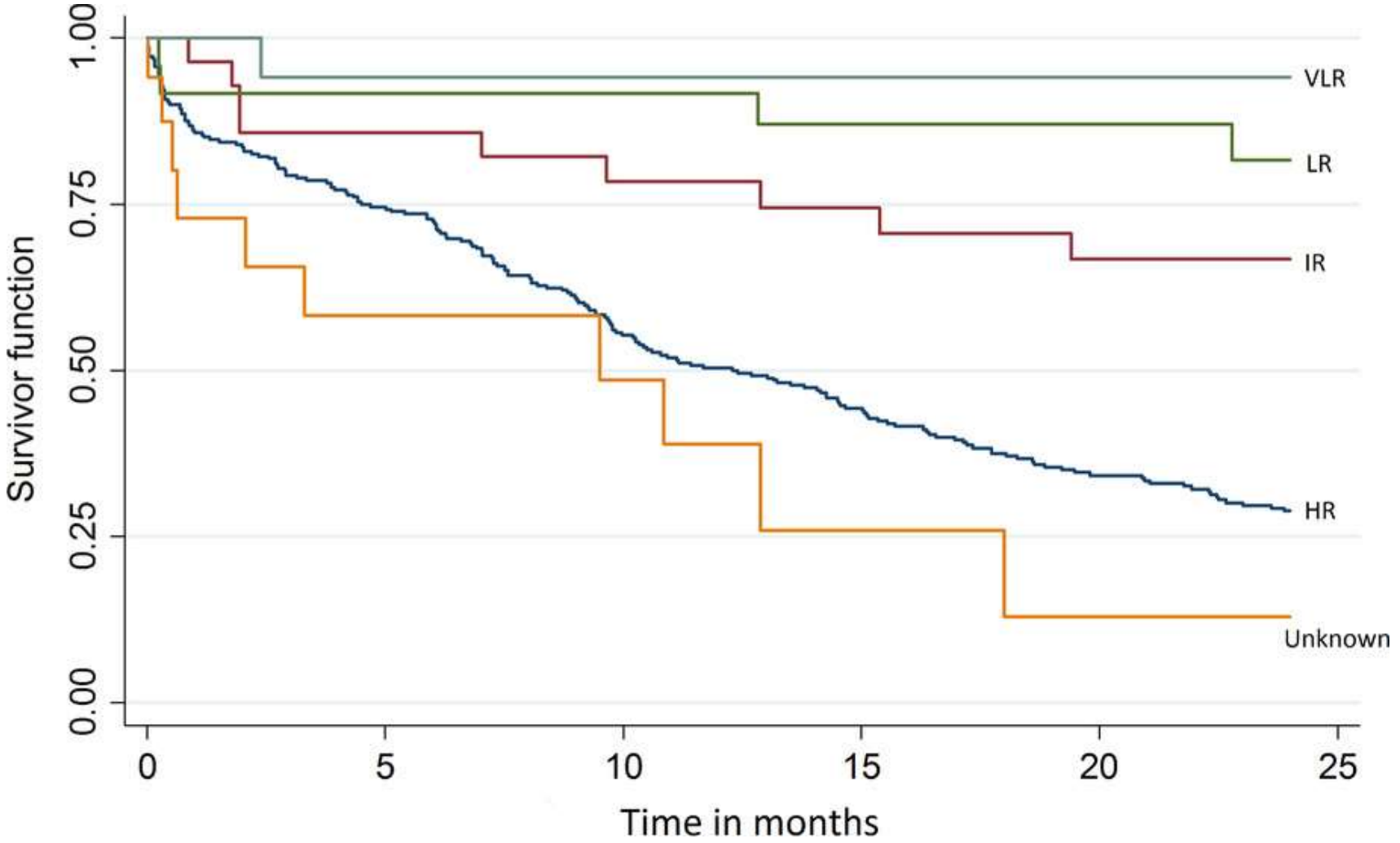

FIGURE 3. Kaplan–Meier curves of the two-year OS for risk classification ($P < 0.001$)

VLR, very low risk; LR, low risk; IR, intermediate risk; HR, high risk

3.4. Histology and tumour markers *(see Table 1 and Supplemental Table S4)*

NB was confirmed by core biopsy in 270 patients (53.3%), with 128 (31.8%) classified as unfavourable histology and 80 (20.5%) as favourable histology. Sixty-two (15.9%) were diagnosed by FNA without sufficient information to distinguish between favourable or unfavourable histology. The diagnosis in the remaining 120 (30.8%) patients was made through bone marrow cytology and/or imaging with raised urine catecholamine levels. Patients with favourable histology, according to the International Neuroblastoma Pathology Classification (INPC), had a superior two-year OS of 66.6% whilst those with unfavourable histology fared worse at 32.8% (p = 0.002, 95% CI).

In patients with HR disease, the mean LDH level (n = 251, 78.7%) was 2 364 UI/L (median 1 197, 255-11 520) and for those with IR disease (n = 27, 8.4%) and LR disease (n = 20, 6.3%), it was 930 UI/L (median 595, 184-1 787) and 498 UI/L (median 407, 241-806), respectively (p < 0.01, 95% CI). In patients with HR disease, the serum ferritin (n = 189, 80.4%) had a mean of 483 ng/dl (median 263, 18-1 650) and in those with IR disease (n = 18, 7.7%) and LR disease (n = 15, 6.4%), it had mean values of 97 ng/dl (median 239, range 37-342) and 64 ng/dl (median 244, range 15-440), respectively (p < 0.01, 95% CI). Patients with an LDH value < 750 U/L at diagnosis had a more favourable two-year OS of 54.8% versus 25.2% for an LDH value > 750 U/L (p < 0.0001, 95% CI), and those with ferritin levels < 120 ng/dl at diagnosis had a more favourable two-year OS of 66.3% compared to levels > 120 ng/dl (25.6%) (p = 0.0004, 95% CI).

MYCN was amplified in 54% (n = 78) of patients. Patients with MYCN-non-amplified tumours (n = 64, 44%) had a two-year OS of 51.3% compared to 37.3% for patients with MYCN-amplified tumours while patients with a copy number gain (1-9 copies,

n = 3, 2%) of MYCN had the best two-year OS of 66.6% (p = 0.002, 95% CI). Raised urine catecholamine levels were detected in 81.6% (n = 221) of patients. Patients with raised catecholamine levels at diagnosis had a poorer two-year OS of 34.9% in comparison to 63.4% in patients with normal urine values (p = 0.05, 95% CI). Patients with raised urine catecholamine levels at diagnosis had a poorer two-year OS of 34.9% as opposed to 63.4% in patients with normal levels (p < 0.049).

3.5. Nutritional status at diagnosis and outcome (see Supplemental Table S5)

Nutritional status was significant as patients with a weight for age (WFA) above the -1SD z-score (n = 162, 60%) had a better two-year OS of 86.6% than wasted patients (WFA < -2SD z-score, n = 55, 20.4%) at 79.3% (p = 0.04). Patients with a height for age (HFA) above the -1SD z-score (n = 129, 49.8%) had a better two-year OS of 88.1% than stunted patients (HFA < -2SD z-score, n = 83, 32%) at 84.1% (p = 0.05).

3.6. Multivariate analysis (see Table 2)

On multivariate analysis, age > 18 months (p = 0.03, HR 0.96, 95% CI, 1.01-1.3), LDH > 750 U/L (p < 0.0001, HR 0.96, 95% CI, 1.3-1.9), ferritin > 120 ng/dl (p = 0.02, HR 0.96, 95% CI, 1.01-1.5) and MYCN gene amplification (p = 0.01, HR 0.96, 95% CI, 1.03-1.3) were significant covariates. Stage (p < 0.0001, HR 0.89, 95% CI, 1.6-3.5) and risk classification (p < 0.0001, HR 0.96, 95% CI, 1.4-2.1) were also significant. Pathology according to the INPC was not a significant covariate (p = 0.37, HR 0.96, 95% CI, 0.8-1.3).

3.7. Management protocols (see Table 3)

Two patients died before treatment was started (see flow diagram). Only 253 (64.9%) patients were treated with curative intent although 354 (90.8%) patients received

TABLE 3: Treatment (univariate analysis)				
Chemotherapy				
Chemotherapy interruptions	**Number of patients**	**Percentage**	**Two-year OS**	**p-value CI = 95%**
Never started	2	< 1%		
Surgery only	24	6.2%		
Insufficient data	10	2.7%		
Chemotherapy received	354	90.8%		
No interruptions	186	52.4%		
Interrupted	168	47.5%		
High-risk protocols				
Rapid COJEC	49	22.17%	24.8%	p = 0.01
OPEC/OJEC	99	44.8%	32.5%	
St Jude NB84	64	28.96%	41%	
Remission rate post induction				
Total evaluated	221			
Remission	68	30.8%		
Not in remission	153	69.2%		
Surgery				
Total	390			
No	216	55.6%		
Yes	174	44.4%		
Complete	98	56.3%	60.4%	< 0.01
Incomplete	76	43.6%	57.3%	
HR				
Total	294			
No surgery	185	62.8%	16.23%	< 0.01
Surgery	109	37.7%		
Incomplete	49	60%	51.4%	
Complete	60	40%%	46.4%	
IR				
Total	30			
No surgery	9	30%	42.8%	< 0.01
Surgery	21	70%		
Incomplete	14	66.6%	70.1%	
Complete	7	33.3%	83.3%	
LR				
Total	26			
No surgery	4	16.6%	42.8%	< 0.01
Surgery	22	83.4%		
VLR				
Total	17			
No surgery	2	11.8%	100%	< 0.01
Surgery	15	88.2%		
Radiotherapy				
Total	390			
Yes	61	15.6%	44.8%	< 0.01
No	329	84.4%	36.2%	
HR				
Total	224			
Radiotherapy	37	16.5%	42.4%%	< 0.01

No radiotherapy	187	83.5%	26.1%	
IR				
Total	11			
Radiotherapy	1	9%	75%	< 0.01
No radiotherapy	10	91%	63.3%	
Surgery and radiotherapy				
Total HR	167			
No surgery without radiotherapy	153	91.6%	17.3%	< 0.01
No surgery with radiotherapy	14	8.4%	25.8%	< 0.01
OS – overall survival; VLR – very low risk; LR – low risk; IR – intermediate risk; HR – high risk				

chemotherapy. Surgery as only treatment was received by 24 patients (6.2%) for LR disease. Complete excisions or debulking procedures were performed only in 174 (44.4%) patients (including 23 LR patients). In the IR, LR and VLR groups, 11 (36.6%), four (16.6%) and two (11.8%) patients respectively were not operated on. VLR patients had a two-year OS of 100% independent of surgical status. Tumours completely resected for IR and LR patients respectively had an improved two-year OS of 83.3% and 84.4% versus a two-year OS of 42.8% and 42.8% ($p < 0.01$, 95% CI) for non-operated tumours. In the HR group, 185 (62.8%) patients were assessed as inoperable. Not all HR patients were operated on, and complete resection in HR patients (n = 60, 40%) had a two-year OS of 46.4% versus non-operated tumours (n = 185, 62.8%) at 16.2% ($p < 0.01$, 95% CI).

Different institutions used different chemotherapy protocols in HR disease. OPEC/OJEC (n = 99, 44.8%), St Jude NB84 (n = 64, 28.9%) and Rapid COJEC (n = 49, 22.2%) were the three main protocols, and 28.1% of the patients were treated with several other protocols. The post-induction remission rate for HR patients was 30.8%. The two-year OS for OPEC/OJEC was 32.5% compared to 41% for the St Jude protocol and 24.8% for Rapid COJEC ($p = 0.05$, 95% CI). (37%).

Patients who received radiotherapy to the primary tumour site had an improved outcome with a two-year OS of 44.8% compared to those without radiotherapy treatment with an OS of 36.2% ($p < 0.01$, 95% CI). HR patients who received radiotherapy treatment had a two-year OS of 42.4% compared to those without radiotherapy treatment with an OS of 26.1% ($p < 0.01$, 95% CI). Patients with HR disease who never came for surgery but who did receive radiotherapy had a superior two-year OS of 25.8% as opposed to 17.3% for those who did not receive either surgery or radiotherapy ($p < 0.01$, 95% CI).

Of the 295 HR patients, only 11 (3.7%) *(see Supplemental Table 6)* received a single autologous stem cell transplant with a significant two-year OS of 72.7% (p = 0.029, 95% CI). Only 13 (33.3%) patients had therapeutic MIBG treatment for refractory disease (n = 12) or relapse (n = 1) with a non-significant two-year OS of 38.4% (p = 0.4917, 95% CI). Cessation of all treatment occurred in 168 (47.5%) patients due to either disease progression or refractory disease.

3.8. Follow-up (*see Supplemental Tables 7 and 8)*

The median survival time of all patients was 13 months (0.1-209 months): for HR patients it was 7.5 months (0.1-87 months), for IR patients it was 116 months (36-193 months), for LR patients it was 140 months (76-199 months) and for VLR patients it was 123 months (36-209 months).

The reason for death was documented in 260 patients of whom 246 (94.6%) were disease related. The majority of HR patients (n = 153; 58.9%) had progressive disease (PD) while nine (3.1%) relapsed. For those with IR disease, 11 (36.7%) had PD and three (10%) relapsed while for those with LR disease, three (11.5%) had PD and three (11.5%) relapsed. Only one patient with VLR disease had PD.

Treatment toxicity contributed to 3.2% (n = 10) of the deaths whereas surgical complications contributed to 0.6% (n = 2) of the deaths. Ten patients (2.6%) died secondary to neutropenic sepsis and two (0.6%) to cardiotoxicity.

The time of death was documented in 56.7% of patients with 95 deaths (24.4%) occurring within six months after diagnosis. A total of 88.7% of deaths occurred within two years from diagnosis.

Of the total cohort, 50 (12.8%) patients were lost to follow-up, constituting 40% of surviving patients ($p < 0.001$, 95% CI), with 54% being HR, 10% being IR, 10% being LR and 6% being VLR patients.

4. Discussion

This study represented the first documented multicentre retrospective cohort study of NB in sub-Saharan Africa. Although the majority of cases were diagnosed in the 18-60-months age group, similar to international trends, the median age was higher at 39.9 months compared to 19 months in the United States of America (HIC) or 33.2 months in Egypt (MIC).[20]

South Africa's healthcare system suffers from various resource challenges in the management of NB. Diagnostic imaging and staging were done in the patients under study, ranging from less sensitive to specific imaging. Plain X-rays are nonspecific with limited diagnostic value beyond the silhouette of primary tumours[21] or mixed sclerotic and lytic lesions of metastasis.[22] Skeletal screening was performed by means of various imaging techniques, but only 41.8% of the study group were surveyed by MIBG scan. Nonnuclear techniques such as plain-film X-rays can be utilised at diagnosis but are not sufficiently sensitive to evaluate skeletal treatment response as healing lesions can present as sclerotic lesions, similar to primary lesions.[22] The metabolic response to therapy, demonstrated with MIBG scans, has a higher sensitivity and proven prognostic value.[23]

In our study, Stage 4 patients constituted 70% of the total cohort, which is higher than the 40% reported by the INRG[24] or the 41.9% in European studies.[25] Yet, the incidence of Stage 4 disease was comparable to studies in Indian (71.4%)[26] and Kenyan (92.8%)[27] hospitals. Although lung metastases were present in 3.8% of patients, which

is marginally higher than reported in North America[28], only 76.7% of patients had CT or MR imaging and 41.8% a ^{123}I-MIBG scan to confirm metastasis. The percentage of lung metastasis could be underestimated. Unknown origin of the primary tumour only occurred in 2.1% of patients as opposed to the 10% reported internationally.[23]

Only 53.3% of the diagnoses in this cohort were confirmed by biopsy, similar to MICs such as Egypt with 40-60%[20] but less than Argentina with up to 96%.[29] Diagnoses were made through FNA in 15.9% of patients due to institutional expertise and its being a less invasive technique in emaciated patients with advanced disease. Quite a number of patients were diagnosed through disease-associated imaging, bone marrow aspirates and raised catecholamine levels, which limited the necessary testing for MYCN in 37.1% (n = 245) patients.

The SIOP-PODC guidelines for LDH and ferritin in NB advise 750 UI/L and 120 ng/dl respectively as the threshold values for poor and good prognosis,[2] which was confirmed by our study. Our study indicated that 54% of patients were MYCN amplified, which is higher than the 20% reported internationally.[30] MYCN copy number gains produced conflicting reports with regard to prognosis[31,32] but in this study were associated with a good prognosis. Even with a limited number of tumours' MYCN status known (n = 145, 37%), a two-year OS advantage was demonstrated between MYCN-amplified and MYCN-non-amplified patients (p = 0.002). This compares with international trends.[2]

All major historical HR induction chemotherapy regimens were used in South Africa, according to institutional preference. The South African doxorubicin-containing post-induction CR rate of 38.5% in HR patients was less than in the doxorubicin-containing COG A3973 studies with up to a 66.1% CR rate.[33] The South African OPEC/OJEC

post-induction CR rate of 29.7% was less than in European studies with a 38% CR rate.[16] The South African Rapid COJEC post-induction CR rate of 16.3% was considerably less than the 44% CR rate for Rapid COJEC in Europe.[10]

Optimising local control is important for improved survival and cure.[34] International definitions of complete resection were based on more than 95% or more than 90%.[34,35] In the COG A3973 studies, resection of > 90% had a better five-year OS of 57.3% compared with 49.4% for resection of < 90% (p = 0.3), and in the HR-NBL1/SIOPEN Trial, complete resection had a better five-year OS compared to incomplete resection (39% vs 30%).[36] In our cohort, 62.8% of the HR group had no surgery. Outcomes in resected patients in all risk groups, regardless of the volume of tumour resection, had a better two-year OS (46.4%) compared to those who had no resection at all (two-year OS 16.23%). Various factors contribute to resectability of tumours, but in this multicentre study where surgical skills and experience amongst surgeons differed, it has been shown that, as in international studies, surgery improved survival in patients with undetectable metastatic disease confirmed on bone marrow aspirate and trephine as well as ^{123}MIBG scan.[34]

In keeping with expert opinion in the SIOP-PODC guidelines,[2] in the absence of a surgical resection, radiotherapy to the primary tumour bed, with or without metastatic sites, had a short-term benefit in patients with a superior two-year OS in unresected patients receiving radiotherapy versus patients not receiving radiotherapy.

European studies reported chemotherapy toxicity-related mortality of 3% for OPEC/OJEC and 4% for Rapid COJEC,[37] which is comparable to the 3.2% toxicity-related mortality in this cohort, keeping in mind that a less toxic OPEC/OJEC regimen

was used locally. Yet, it was higher than the 2-2.5% toxicity reported in St Jude studies.[38]

The risk-based two-year OS of the South African cohort was lower when compared to the outcomes reported in HICs (95% for LR disease, 90-95% for IR disease and 50-60% for HR disease)[3] and an MIC such as Argentina.[29] The median survival of 12.2 months was lower than reported in other MICs such as India, China and Brazil.[26,39] Patients with HR disease dominated in this cohort at 75.6%, which was higher in comparison to INRG Task Force studies at 40%.[24] A North American study concluded that biology was related to race with HR disease more prevalent in black patients, possibly explaining the finding.[40] A combination of late presentation and poor disease biology possibly explains the median time to death of six months from diagnosis for 42% of patients. Malnutrition was a significant risk factor in our cohort, as reported in previous international studies.[41]

Limitations included the retrospective data analysis of prospectively collected data, where collection might not have been uniform. There might also be a lack of documentation of crucial findings. The limited access to genetic assessments such as DNA ploidy, MYCN gene amplification and 1p36 deletion status might have impacted negatively on risk stratification determination. Nonstandard documentation of management information, investigation reporting definitions, surgical classifications and treatment outcomes limits comparative studies due to the heterogeneous nature of the data. During the study period the availability of investigations, like isotopes for nuclear imaging for staging and risk classification was not always guaranteed. This could have contributed to staging and risk classification being inaccurate, resulting in suboptimum treatment and poorer outcomes. The treatment protocols were not standardised nationally. Treatment was adapted with limitations of accurate risk

classification according to the clinical presentation and with palliative or curative intent. No accurate longitudinal comparisons could therefore be made.

In resource-constrained settings, cost-effective management options are of great importance.[42] Even with limitations in diagnostic procedures, referral and treatment protocols and the variable availability of expertise in all fields, management should be optimised to obtain the best possible outcomes. Our study indicated that non-doxorubicin-containing and doxorubicin-containing chemotherapy produced comparable outcomes. OPEC/OJEC is an acceptable primary protocol, especially considering the favourable toxicity profiles, while attempting surgery. Even incomplete surgical resections have a survival advantage versus no surgical intervention.

5. Conclusions

The outcomes for NB patients varied according to different treatment protocols used in South Africa. A uniform, nationally standardised treatment strategy might ensure better risk classification, ensure the appropriate intensity of chemotherapy, especially in LR and IR disease. Harmonising treatment might improve OS by creating equitable access to all treatment modalities, including surgical expertise, autologous stem cell transplant and maturation therapy. To this end, a standardised protocol, incorporating risk-based therapy, should be implemented.

Conflict of interest

The authors have no conflict of interest to disclose.

Data availability statement

The data that supports the findings of this stuyd are available on request from the the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Acknowledgements

Dr Van Heerden acknowledges the Department of Paediatric Haematology and Oncology, Antwerp University Hospital, University of Antwerp, for research support with financial contributions from the VZW Kinderkankerfonds, Belgium.

The authors acknowledge the SACCSG for supporting the study and the SACTR for providing statistical data.

References

1. Matthay K, Maris J, Schleiermacher G, et al. Neuroblastoma. ***Nat Rev Dis Primers.*** 2016; 2:16078.
2. Parikh N, Howard S, Chantada G, et al. SIOP-PODC adapted risk stratification and treatment guidelines: Recommendations for neuroblastoma in low- and middle-income settings. ***Pediatr Blood Cancer.*** 2015;62(8):1305-1316.
3. Pinto N, Applebaum M, Volchenboum S, et al. Advances in risk classification and treatment strategies for neuroblastoma. ***J Clin Oncol.*** 2015;33(27):3008-3017.
4. Whittle S, Smith V, Doherty E, et al. Overview and recent advances in the treatment of neuroblastoma. ***Expert Rev Anticancer Ther.*** 2017;17(4):369-386.
5. Yáñez Y, Grau E, Rodríguez-Cortez VC, et al. Two independent epigenetic biomarkers predict survival in neuroblastoma. ***Clin Epigenetics*****.** 2015;7(1):16.
6. Stuckler D, Basu S, McKee M. Health care capacity and allocations among South Africa's provinces: Infrastructure-inequality traps after the end of apartheid. ***Am J Public Health.*** 2011;101(1):165-172.
7. [World Bank Group Web site] 2018. Available at: https://data.worldbank.org/income-level/low-and-middle-income. Accessed 12 Nov 2018.
8. Israels T, Ribeiro RC, Molyneux EM. Strategies to improve care for children with cancer in sub-Saharan Africa. ***Eur J Cancer.*** 2010;46(11):1960-1966.
9. Mayosi B, Lawn J, Van Niekerk A, et al. Health in South Africa: Changes and challenges since 2009. ***Lancet.*** 2012;380(9858):2029-2043.

10. Stefan D, Stones D, Wainright R, et al. Childhood cancer incidence in South Africa, 1987–2007. ***S Afr Med J.*** 2015;105(11):939-947.
11. Mayosi B, Benatar S. Health and Health Care in South Africa — 20 Years after Mandela. ***New Engl J Med***. 2014 ; 371(14) : 1344–1353.
12. Benatar S. The challenges of health disparities in South Africa. ***S Afr Med J***. 2013 ; 103(3):154-155.
13. Brodeur G, Pritchard J, Berthold, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. ***J Clin Oncol.*** 1993;11(8):1466-1477.
14. Park J, Bagatell R, Cohn S, et al. Revisions to the International Neuroblastoma Response Criteria: A consensus statement from the National Cancer Institute Clinical Trials Planning Meeting. ***J Clin Oncol.*** 2017;35(22):2580-2587.
15. Eisenhauer E, Therasse P, Bogaert J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). ***Eur J Cancer.*** 2009;45(2):228-247.
16. Peinemann F, Tushabe D, Berthold F. Rapid COJEC versus standard induction therapies for high-risk neuroblastoma. ***Cochrane Database Syst Rev.*** 201519(5):CD010774. doi: 10.1002/14651858.CD010774.pub2.
17. Bowman L, Hancock M, Santana V, et al. Impact of intensified therapy on clinical outcome in infants and children with neuroblastoma: The St Jude Children's Research Hospital Experience, 1962 to 1988. ***J Clin Onco*****.** 1991;9(9):1599-1608.
18. Stram D, Matthay K, O'Leary M, et al. Consolidation chemoradiotherapy and autologous bone marrow transplantation versus continued chemotherapy for

metastatic neuroblastoma: A report of two concurrent Children's Cancer Group studies. ***J Clin Oncol.*** 1996;14(9):2417-2426.

19. Coze C, Hartmann O, Michon J, et al. NB87 induction protocol for Stage 4 neuroblastoma in children over 1 year of age: A report from the French Society of Pediatric Oncology. ***J Clin Oncol.*** 1997;15(12):3433-3440.
20. Al-Tonbary Y, Badr M, Mansour A, et al. Clinico-epidemiology of neuroblastoma in north east Egypt: A 5-year multicenter study. ***Oncol Lett.*** 2015;10(2):1054-1062.
21. Duarte M, Gelmini A, Marques de Souza G. Neuroblastoma: A correlation between radiologic methods. ***Urol Nephrol Open Access J.*** 2018;6(2):65-67.
22. David R, Lamki N, Fan S, et al. The many faces of neuroblastoma. ***RadioGraphics.*** 1989;9(5):859-882.
23. Decarolis B, Hero B, Simon T, et al. Iodine-^{123}meta-iodobenzylguanidine scintigraphy scoring allows prediction of outcome in patients with Stage 4 neuroblastoma: Results of the Cologne Interscore Comparison Study. ***J Clin Oncol*.** 31(7):944-951.
24. Cohn L, Pearson A, London W, et al. The International Neuroblastoma Risk Group (INRG) classification system: An INRG Task Force report. ***J Clin Oncol.*** 2009;27(2):289-297.
25. Haupt R, Garaventa A, Gambini C, et al. Improved survival of children with neuroblastoma between 1979 and 2005: A report of the Italian Neuroblastoma Registry. ***J Clin Oncol.*** 2010;28(14):2331-2338.
26. Bansal D, Marwaha R, Trehan A, et al. Profile and outcome of neuroblastoma with conventional chemotherapy in children older than one year: A 15-years' experience. ***Indian Pediatrics*** 2008;45:135-139.

27. Kitonyi G, Macharia W, Mwanda O, Pamnani R. Clinico-pathologic characteristics and treatment outcomes in children with neuroblastoma at the Kenyatta National Hospital, Nairobi. ***East Afr Med J.*** 2009;86(12 suppl):S39-S46.
28. DuBois S, London W, Zhang Y, et al. Lung metastases in neuroblastoma at initial diagnosis: A report from the International Neuroblastoma Risk Group (INRG) Project. ***Pediatr Blood Cancer*** 2008;51(5):589-592.
29. Moreno F, Lopez Marti J, Palladino M, et al. Childhood neuroblastoma: Incidence and survival in Argentina. Report from the National Pediatric Cancer Registry, ROHA Network 2000-2012. ***Pediatr Blood Cancer*** 2016;63(8):1362-1367.
30. Smith V, Foster J. High-risk neuroblastoma treatment review. ***Children*** 2018;5(9):114. doi:10.3390/children5090114.
31. Wang M, Zhou C, Cai, R et al. Copy number gain of MYCN gene is a recurrent genetic aberration and favorable prognostic factor in Chinese pediatric neuroblastoma patients. ***Diagnostic Pathology*** 2013;8(5):1-6.
32. Seeger R, Brodeur G, Sather H, et al. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. ***N Engl J Med*****.** 1985;313(18):1111–1116.
33. Yanik G, Parisi M, Shulkin B, et al. Semiquantitative mIBG scoring as a prognostic indicator
in patients with Stage 4 neuroblastoma: A report from the Children's Oncology Group. ***J Nucl Med.*** 2013;54(4):541-548.

34. Von Allmen D, Davidoff A, London W, et al. Impact of extent of resection on local control and survival in patients from the COG A3973 study with high-risk neuroblastoma. ***J Clin Oncol.*** 2017;35(2):208-216.
35. Kiely E. A technique for excision of abdominal and pelvic neuroblastoma. ***Ann R Coll Surg Engl.*** 2007;98(4):342-348.
36. Holmes K, Poetschger U, Sarnacki S, et al. The influence of surgical excision on survival in high-risk neuroblastoma revisited after introduction of ch14.18/CHO immunotherapy in the HR-NBL1/SIOPEN trial. ***J Clin Oncol.*** 2018;36(15 suppl):10521-10521.
37. Pearson A, Pinkerton C, Lewis I, et al. High-dose rapid and standard induction chemotherapy for patients aged over 1 year with Stage 4 neuroblastoma: A randomised trial. ***Lancet Oncology*** 2008;9(3):247-256.
38. Bowmen L, Hancock M, Santana V, et al. Impact of intensified therapy on clinical outcome in infants and children with neuroblastoma: The St Jude Children's Research Hospital Experience, 1962 to 1988. ***J Clin Oncol.*** 1999;9:1599-1608.
39. Parize I, Haddad B, Cavalli L, et al. Neuroblastoma in southern Brazil: An 11-year study. ***J Pediatr Hematol Onco.*** 2006;28(2):82-87.
40. Henderson TO, Bhatia S, Pinto N, et al. Racial and ethnic disparities in risk and survival in children with neuroblastoma: A Children's Oncology Group study. ***J Clin Oncol.*** 2011;29(1):76-82.
41. Bauer J, Jürgens H, Frühwald M. Important aspects of nutrition in children with cancer. ***Adv Nutr.*** 2011;2(2):67-77.
42. Bhakta N, Martiniuk A, Gupta S, et al. The cost effectiveness of treating paediatric cancer in low-income and middle-income countries: a case-study

approach using acute lymphocytic leukaemia in Brazil and Burkitt lymphoma in Malawi**.** ***Arch Dis Child*** 2012; .98(2), 155–160.

APPENDIX A: COG classification

INSS	Age	MYCN	Shimada	DNA ploidy	Risk group
1	Any	Any	Any	Any	Low
2A/2B	< 365 d	Any	Any	Any	Low
	> 365 d	Non-amplified	Any	-	Low
	> 365 d	Amplified	Favourable histology	-	Low
	> 365 d	Amplified	Unfavourable histology	-	High
3	< 365 d	Non-amplified	Any	Any	Intermediate
	< 365 d	Amplified	Any	Any	High
	> 365 d	Non-amplified	Favourable histology	-	Intermediate
	> 365 d	Non-amplified	Unfavourable histology	-	High
	> 365 d	Amplified	Any	-	High
4	< 548 d	Non-amplified	Any	Any	Intermediate
	< 548 d	Amplified	Any	Any	High
	> 548 d	Any	Any	-	High
4S	< 365 d	Non-amplified	Favourable histology	> 1	Low
	< 365 d	Non-amplified	Any	1	Intermediate
	< 365 d	Non-amplified	Unfavourable histology	Any	Intermediate
	< 365 d	Amplified	Any	Any	High

APPENDIX B: INRG classification

INRG stage	Age (months)	Histologic category	Grade differentiation	MYCN	11 q aberration	Ploidy	Pretreatment risk group
L1/L2	Any	GN maturing GNB intermittent	Any	Any	Any	Any	A – Very low risk
L1	Any	Any, except GN maturing GNB intermittent	Any	nAmp	Any	Any	B – Very low risk
				Amp	Any	Any	K – High risk
L2	< 18	Any, except GN maturing GNB intermittent	Any	nAmp	No	Any	D – Low risk
					Yes	Any	G – Intermediate risk
	≥ 18	GNB nodular, neuroblastoma	Differentiated	nAmp	No	Any	E – Low risk
					Yes		H – Intermediate risk
			Poorly differentiated or undifferentiated	nAmp	Any		
			Any	Amp	Any	Any	H – High risk
M	< 18	Any	Any	nAmp	Any	Hyperdiploid	F – Low risk
	< 12	Any	Any	nAmp	Any	Diploid	I – Intermediate risk
	12 to < 18	Any	Any	nAmp	Any	Diploid	J – Intermediate risk
	< 18	Any	Any	Amp	Any	Any	O – High risk
	≥ 18	Any	Any	Any	Any	Any	P – High risk
MS	< 18	Any	Any	nAmp	No	Any	C – Very low risk
					Yes	Any	O – High risk
				Amp	Any	Any	R – High risk