Insight in the older neuroblastoma patient

A clinical and molecular characteristics overview

Paediatric oncology

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Abbreviations

EFS	Event-free survival
GNB	Ganglioneuroblastoma
i.e.	id est
INRGSS	International Neuroblastoma Risk Staging System
IQR	Interquartile range
NB	Neuroblastoma
NGS	Next-generation sequencing
No.	Number
NCA	Numerical Chromosomal Aberrations
MIBG	123I-metaiodobenzylguanidine scintigraphy
MYCN	*myc-N*
MC	Medical Centre
OS	Overall survival
PMC	Princess Maxima Center
PALGA	Pathologisch Anatomisch Landelijk Geautomatiseerd Archief
SNP	Single Nucleotide Polymorphism
SCA	Segmental Chromosomal Aberrations
±; SD	Standard deviation
vs.	Versus
WES	Whole Exome Sequencing

Abstract

Background: Neuroblastoma is a malignant tumour and one of the most common extracranial malignant solid tumours in childhood. It most commonly affects children aged ≤5 years, though it may rarely occur in older children, with 10% of cases ≥6 years. There appear to be differences in age-related groups. We combined clinical factors and molecular characteristics, which provides a comprehensive overview. **Methods:** Data was conducted within a multi-database system: Radboudumc and Emma Children's Hospital from 2008 till 2014; Princess Maxima Center from 2014 till April 2023. Pathological and clinical records were obtained for molecular characteristics. We reviewed the medical records for clinical presentation, biochemical studies, treatment, and follow-up survival. **Results:** Higher proportion of female individuals were diagnosed with neuroblastoma (55.8% vs. 47.7%). Our study indicated a higher proportion of patients classified as high risk (83.7%). Our study revealed lower overall survival rates compared to those reported in the literature for both intermediate-risk disease (83.3% vs. >90%) and high-risk patients (41.9% vs. >50%). Immunotherapy was significantly associated with overall survival (p=0.008). We have found MYCN mutations in 16.3% of the cases. The LOH1p was close to significantly associated with overall survival (p=0.058) and event-free survival (p=0.085). The overall survival was not significantly associated with Risk groups (p=0.162), ATRX mutations (p=0.246), gain of 17q (p=0.250), age above 10 (p=0.296). **Conclusion:** These findings suggest a potentially unfavourable prognosis for patients aged six years and above. This study initiates a comprehensive overview of the patient population, setting the stage for future research to build upon.

Keywords: *neuroblastoma; paediatric oncology; older children; adolescent.*

Introduction

Neuroblastoma (NB) is a malignant tumour, which arises from the neural crest cells of the sympathetic nervous system. This results in tumours in the adrenal glands and paravertebral ganglia. It is one of the most common extracranial malignant solid tumours in childhood and accounts for 7% of all childhood malignancies.(1) It is often a lethal cancer of early childhood that accounts for 10% of paediatric cancer mortality.(2) It most commonly affects children aged 5 years or younger, though it may rarely occur in older children, with 10% of cases ≥6 years(3) and less than 5% of cases diagnosed in children and adolescents ≥10 years.(4)

Older neuroblastoma patients may have different characteristics and a different neuroblastoma behaviour. For example, most malignant tumours have amplification of the MYCN oncogene (encoding the transcription factor N-MYC).(5) In childhood cases, this amplification is found in 25% of neuroblastoma diagnoses. MYCN is usually associated with poor event-free and overall survival, even if the disease is localized.(6) Previous research showed that adolescents with neuroblastoma showed lower quantity of *myc-N* amplification than in childhood cases.(7) Also, in older patient cases, loss of function mutations of ATRX had a relatively high incidence compared to young infants. Additionally, ATRX is frequently mutated in high-risk patients with a poor prognosis.(8) Patients with ATRX mutant neuroblastoma were typically adolescents with an indolent or chronic progressive form of this disease.(9, 10) This phenomenon of chronic NB is also rare with MYCN-amplified disease.(11)

In the young infants with a favourable biology, many of the tumours regress spontaneously, without any treatment required, even in cases of metastatic disease. In contrast to children >18 months at diagnosis, with potential metastatic disease, unfavourable biology or unresectable tumour, where intensive therapy is needed, and overall survival is much lower.(12)

There appear to be differences in age-related groups, but a good overview has not yet been presented. In this paper we would like to provide more clarity in this regard. We will combine clinical factors and molecular characteristics, which provides a more comprehensive picture. We hereby adhere to the limit of over 6 years of age, because the occurrence of NB in adolescents and children over 6 years of age is rare, and few clinical studies are published in this age group.

Also, since the incidence is low at this age, no specific treatment has yet been created for this population. Currently, we are still assuming a similar treatment as for younger children. In this study, we examined characteristics to explore new approaches to therapy.

In conclusion, we investigated the clinical and molecular characteristics of adolescent onset (≥6 years) neuroblastoma.

Methods

Study setting

This study is an analysis of the Molenaar/Tytgat Researchgroups at Princess Maxima Center and was conducted within a multi-database system. Data for the primary outcome was included from Princess Maxima Center from 2014 till April 2023. Few patients were included from Emma Children's Hospital before this time period, from 2008 till 2014.

A request for further molecular data has been submitted to PALGA i.e. the pathologist database. Additionally, a second request for international data of basic features has been submitted to INRG, who maintain an international database for neuroblastoma. The data from these sources will be analysed at a later stage as this has not yet been received.

Study population

Patients were included if diagnosed with histopathological confirmed neuroblastoma ≥6 years of age. Archived pathologic and clinical records were obtained for molecular characteristics. We reviewed the medical records for the clinical presentation, biochemical studies, treatment, and follow-up.

Patients were excluded with a ganglioneuroma, because this is a benign diagnosis. This patient group often has a wait-and-see policy and a good prognosis. Therefore, we prefer not to compare them with each other.

Outcomes

Our primary outcome will be the clinical factors and molecular characteristics of the patients included in Princess Maxima Center. Details concerning the molecular characteristics are elaborated in Appendix 2. FISH is not included in the analysis, as it only reflects MYCN, which was already known from other molecular tests. Clinical characteristics will be described in baseline table (See Table 1) and include survival rates and occurrence of events. For the results we will use a time frame of 5 years.

We will include the pathological characteristics of the PALGA database. Finally, we will look at the basic characteristics known from the international database (INRG).

Statistical analysis

Descriptive statistics will be used to evaluate baseline characteristics. Continuous data will be tested for normality. Continuous data with a normal distribution will be described by means and standard deviation. Continuous data with no normal distribution will be compared by Mann-Whitney U test. Non-parametric variables will be described by using the median and quartiles. For categorical variables, frequencies and percentages will be used.

For the statistical analysis IBM SPSS Statistics version 29 will be used. P-values < 0.05 are considered significant.

Results

Study enrolment flowchart

A total of 458 patients were screened for inclusion. Of these patients, 413 were excluded because of age <6 years. Furthermore, 2 patients did not meet the in- and exclusion criteria because of diagnosis of ganglioneuroma. A total of 43 patients' records were included for analysis. (Figure 1)

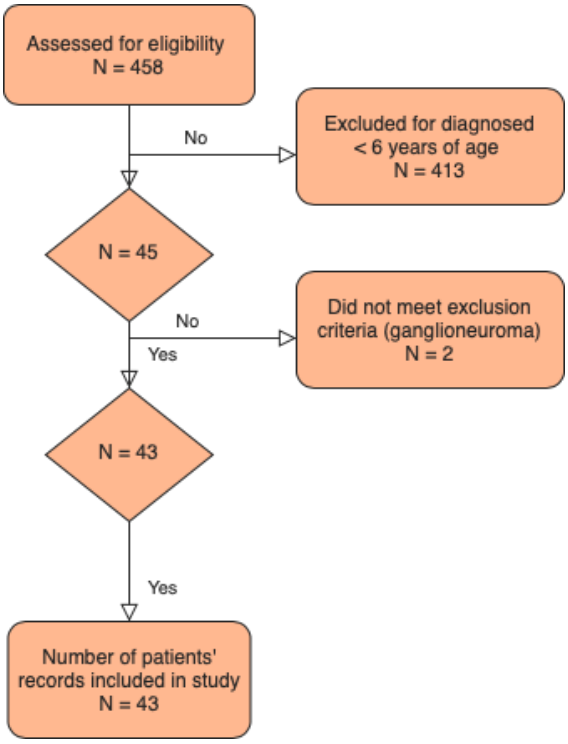


Figure 1. Patient inclusion and exclusion flowchart.

Baseline patient characteristics

In this study, we included 43 patients of whom 44.2% male and 55.8% female. The median age was 7.81 [IQR 6.42-11.08] years. Of the 43 patients, 90.7% had a diagnosis of neuroblastoma, the remaining patients had a diagnosis of ganglioneuroblastoma.

Table 1. Baseline characteristics

Baseline characteristics	Statistics		Total (N=43)
Age at diagnosis (yrs)	Median [IQR]		7.81 [6.42 – 11.08]
Sex (male)	N (%)		19 (44.2)
Ethnicity	N (%)	Caucasian Other Unknown	25 (58.1) 5 (11.6) 13 (30.2)

Diagnosis	N (%)	NB GNB	39 (90.7) 4 (9.3)
Histology	N (%)	NB, undifferentiated NB, poorly differentiated NB, differentiating GNB, intermixed GNB, nodular NB, unclassified	8 (18.6) 12 (27.9) 4 (9.3) 3 (7.0) 1 (2.3) 15 (34.9)
INRS	N (%)	Stage 1 Stage 2 Stage 3 Stage 4	1/42 (2.4) 1/42 (2.4) 5/42 (11.9) 35/42 (83.3)
INRG staging system*	N (%)	L1 L2 M MS	1 (2.3) 6 (14.0) 36 (83.6) 0 (0)
Riskgroup		OG MRG HRG Other/switch**	1 (2.3) 6 (14.0) 31 (72.1) 5 (11.6)
Location tumour	N (%)	Adrenal gland left Adrenal gland right Abdominal, side chain Pelvic Thoracic Cervical	10/42 (23.8) 18/42 (42.9) 6/42 (14.3) 4/42 (9.5) 5/42 (11.9) 1/42 (2.4)
Distant metastasis	N (%)		34 (79.1)
Location metastasis	N (%)	Osteomedullary Distant lymph nodes Lung Liver Skin Brain	30 (69.8) 9 (20.9) 1 (2.3) 3 (7.0) 0 (0) 0 (0)
MIBG	N (%)	+ +/-*** -	20/26 (76.9) 2/26 (7.7) 4/26 (15.4)
Surgery	N (%)		26 (60.5)
Chemotherapy	N (%)	NBL2009/GPOH NB04 POG SIOPEN HR/NBL/2 Rapid COJEC SIOPEN HR/NBL/2 GPOH induction No chemotherapy	37 (86.0) 1 (2.3) 2 (4.7) 2 (4.7) 1 (2.3)
Immunotherapy	N (%)	Yes Partial treatment No	16/42 (38.1) 5/42 (11.9) 21/42 (50.0)

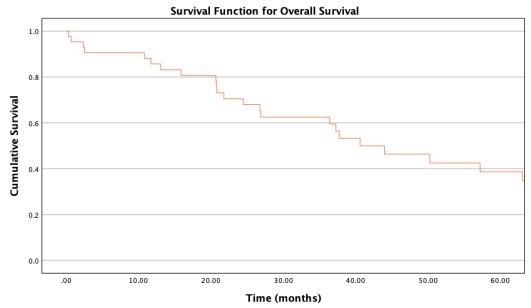
*Algorithm is shown in Appendix 1. (13, 14)

** Cases with upstaged risk after histopathology, before treatment

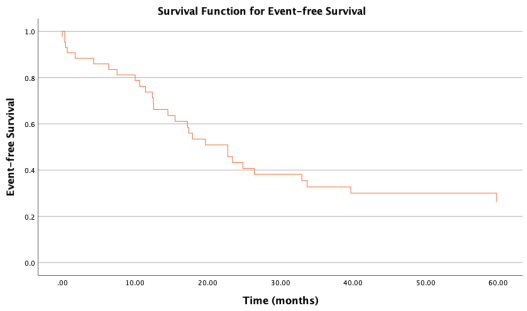
*** MIBG positive and negative tumour parts

IQR = interquartile range; NB = neuroblastoma; GNB = ganglioneuroblastoma; OG = observational group; MRG = medium risk group; HRG = high risk group

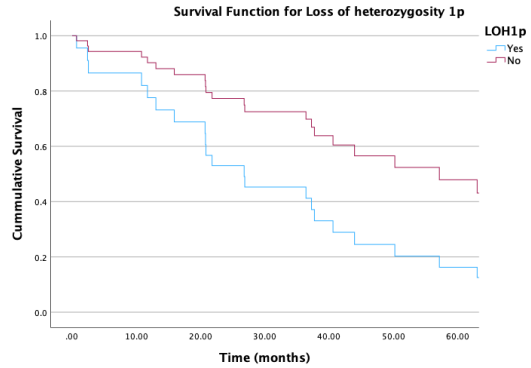
Clinical outcomes



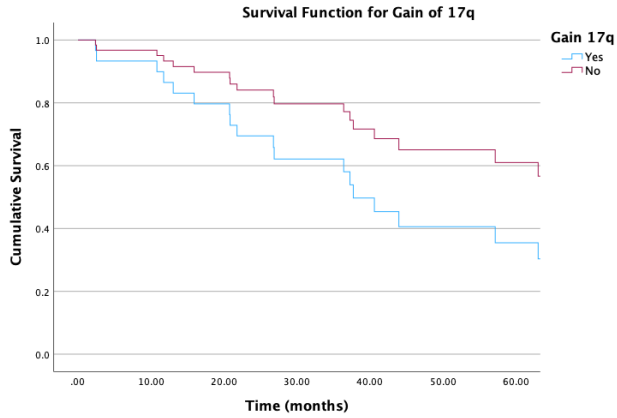
A. Overall survival function.



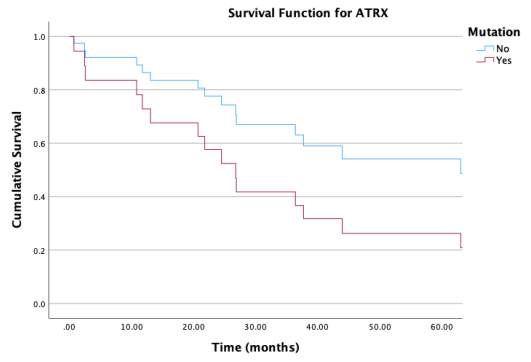
B. Event-free survival function.



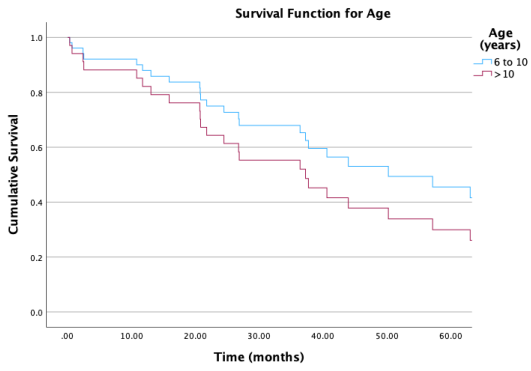
C. Survival function for LOH1p. p=0.058



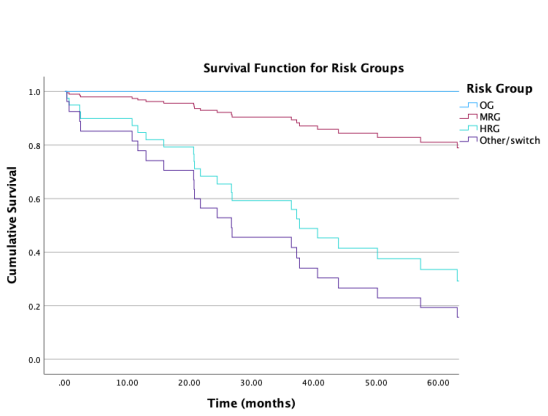
D. Survival function for Gain of 17q. p=0.250



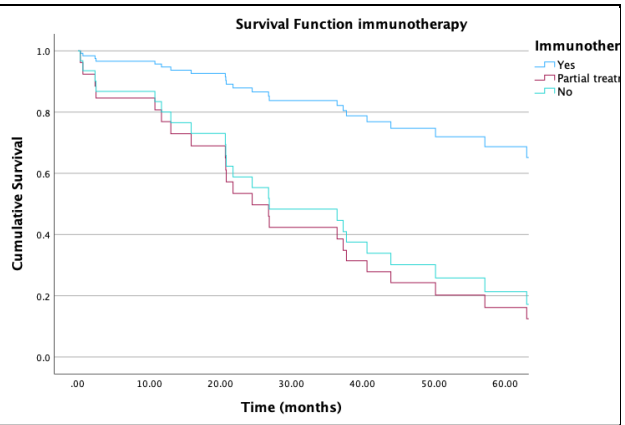
E. Survival function of ATRX mutation. p=0.246



F. Survival function of age divided by the age of 10. p=0.296



G. Survival function divided by risk group. OG=observational group; MRG=medium risk group; HRG=high risk group. p=0.162



H. Survival function for immunotherapy. p=0.008

Figure 2. Survival functions

The overall survival was 32.6% (Figure 2A). The causes of death were resistant disease 6 (14.0%), relapse 15 (34.9%), treatment related toxicity 1 (2.3%) or other causes (direct after first dose chemotherapy) 1 (2.3%). The overall event-free survival was 34.9%. (Figure 2B) The types of events that occurred were relapse 19 (44.2%), progression 6 (14.0%), secondary malignancy 1 (2.3%) and death 2 (4.7%). The loss of heterozygosity 1p was nearly significantly associated with lower overall survival (30.0% vs. 51.6%; HR 0.405; 95% CI 0.160-1.029; p=0.058) (Figure 2C) and event-free survival (10.0% vs. 41.9%; HR 0.488; 95% CI 0.216-1.103; p=0.085) (Appendix 3, Figure 11). The overall survival was not significantly associated with the gain of 17q (40.7% vs. 62.5%; HR 0.477; 95% CI 0.135-1.685; p=0.250) (Figure 2D).

The overall survival was not significantly associated with ATRX mutation (HR 2.178, 95% CI 0.585-8.102, p=0.246) (Figure 2E). Age has been divided by 10 years old to compare for age-related risk (Figure 2F). The overall survival was not significantly associated with age >10 years old. (HR 1.533; 95% CI 0.688-3.417; p=0.296) The medium risk group had an overall survival of 83.3%. The high-risk group had an overall survival of 41.9%. The group that was initially a medium risk case and later had a switch to a higher risk had an overall survival of 20.0% (p=0.162) (Figure 2G). Event-free survival was not significantly associated for risk groups (p=0.388).

Having received immunotherapy was significantly associated with survival. Overall 5-year survival was 68.8% for patients who had completed immunotherapy. Partial treatment cases were patients where therapy was discontinued due to toxicity. There were no surviving

patients in the group who stopped immunotherapy early on. Without treatment the 5-year OS was 25.0% (HR 1.880; 95% CI 1.179-2.996; p=0.008) (Figure 2H).

The survival was not significantly associated with sex (HR 0.982; 95% CI 0.654-1.475; p=0.931), age (HR 1.079; 95% CI 0.984-1.183; p=0.108), MYCN status (HR 1.018; 95% CI 0.534-1.940; p=0.957), ALK (HR 1.227; 95% CI 0.586-2.566; p=0.588). Figures have been shown in Appendix 3.

Molecular outcomes

We have basic genetic abnormalities of all 43 included patients. (Table 2)

Table 2. Basic genetic abnormalities

Basic genetic abnormalities		N = 43 (%)
MYCN status	Normal Amplified Gain	35/42 (83.3) 4/42 (9.5) 3/42 (7.1)
ALK	Normal Gain Mutation	24/30 (80.0) 3/30 (10.0) 3/30 (10.0)
Loss of heterozygosity 1p		10/41 (24.4)
Loss of heterozygosity 11q		12/31 (38.7)
Gain 17q		27/35 (77.1)
ATRX		5/33 (15.2)
SMARCA4		1 (2.3)
TP53		3 (7.0)

Table 3. Cross table Loss of heterozygosity 1p and MYCN status. N (%)

LOH1p	***MYCN***	**Normal**	**Amplified**	**Gain**
	Yes	5 (12.5)	4 (10.0)	1 (2.5)
	No	28 (70.0)	0	2 (5.0)

Table 4. Cross table ATRX and MYCN status. N (%)

ATRX	***MYCN***	**Normal**	**Amplified**	**Gain**
	Yes	4 (12.5)	1 (3.1)	0
	No	23 (71.9)	2 (6.3)	2 (6.3)

Of the 43 patients, we have detailed molecular data (WES/SNP-array/NGS) of 26 of them. Due to the small population of the detailed data, we looked at changes that occurred more than once. The number of patients per genetic test is shown in Table 3. A detailed description of the molecular aberrations can be found in Appendix 2.

Table 5. Number of patients per genetic test

Molecular test	**No. of patients**
WES	6
SNP-array	11
NGS	18

Table 6. Whole Exome Sequencing

*Mutation**	**Total no. Patients**	**Mutation (%)***												
NCA	4	#1 Gain (50)	#7 Gain (100)	#18 Gain (100)										
SCA	6	#2q Partial gain	#4p Partial Loss	#7q Partial gain	#11q Partial loss	#13q Gain	#14q Partial gain	#15q (partial) gain	#16q (Partial) loss	#17p (partial) loss	17q partial gain (66.7)	19p Partial loss	19q (partial) loss	20q (partial) loss
ALK		1 (16.7)												
TP53		2												
NF1	1													
ATRX	1													

*NCA and SCA mutation only shown with n≥2
NCA = Numerical Chromosomal Aberrations; SCA = Segmental Chromosomal Aberrations

Table 7. SNP-Array

*Mutation**	**Total no. patients**	**Mutation (%)**															
NCA	9	#1 Gain (18.2)	#2 Gain (18.2)	#6 Gain (18.2)	#7 Gain (72.3)	#8 Gain (27.3)	#9 Gain (18.2)	#10 Allelic imbalance (18.2)	#12 Gain (18.2)	#17 Gain (27.3)	#18 Gain (45.5)	#20 Gain (18.2)					
SCA	10	#1p Loss (18.2)	#1q Partial gain (36.4)	#2q Partial gain (18.2)	#3p partial loss (27.3)	#4p Partial loss (18.2)	#5q partial gain (27.3)	#6q partial loss (18.2)	#11p allelic imbalance (18.2)	#11q partial loss (36.4)	#12q (partial) gain (27.3)	#13q (partial) gain (45.5)	#15q (partial) gain (36.4)	#17q (partial) gain (18.2)	#19p partial loss (27.3)	#20q partial loss (18.2)	#22q (partial) gain (18.2)
Chromothripsis	1	#17 (9.1)															

*NCA and SCA mutation only shown with n≥2
NCA = Numerical Chromosomal Aberrations; SCA = Segmental Chromosomal Aberrations

Table 8. NGS

Mutation	**No. patients**
No mutation	14
ALK	3
CCND1 amplification	1

Discussion

Summary of findings

This study aimed to examine the clinical and molecular characteristics of neuroblastoma specifically in older children. By focusing on this specific age group, we aimed to gain a deeper understanding of the disease presentation and underlying molecular features in this population. The analysis encompassed comprehensive evaluations of clinical parameters, including demographic data, tumour staging, treatment response, and patient outcomes, as well as molecular profiles, such as genetic alterations, gene expression patterns, and molecular subtypes.

Clinical

During the assessment of baseline characteristics in our study, it was observed that a higher proportion of female individuals were diagnosed with neuroblastoma compared to the reported prevalence (55.8% vs. 47.7%).(15) The incidence is normally slightly more common in boys than in girls, by a ratio of 6:5.(16) However, our results did not reveal any significant disparity in clinical outcomes between male and female patients. This finding is further supported by Figure 4 in Appendix 3, which provides a visual representation of the comparable clinical outcomes among both genders.

Our study revealed a notable prevalence of advanced disease stages in the studied cohort. Specifically, a high proportion of patients were diagnosed with stage 4 (83.3%), stage M (83.6%), and classified as high-risk (72.1%). In contrast, previous research reported a lower proportion of patients older than 5 years with stage 3/4 disease at 75.1%.(17) This finding is in stark contrast to our observed rate of 95.2% for stage 3/4 disease within our population. Furthermore, when considering the classification of patients into risk categories, our study indicated a substantially higher proportion of patients classified as high risk (83.7%) compared to the neuroblastoma population as a whole, where approximately half of the patients are typically classified as high risk.(17)

Moreover, our research revealed lower overall survival rates compared to those reported in the literature for both intermediate-risk disease (83.3% vs. >90%) and high-risk patients (41.9% vs. >50%).(18) These findings suggest a potentially unfavourable prognosis for patients aged six years and above.

Despite the data showing a different survival for risk groups, risk groups were not significantly associated with overall survival (p=0.162) and event-free survival (p=0.388). This may be due in part to a small study population.

Even older children (≥10 years) also seem to have an unfavourable prognosis in comparison to children aged 5-10, but this was not a significant association. Comparable information is described in other literature.(19, 20)

About 90% of neuroblastoma cases are MIBG avid.(21) In alignment with these findings, our study revealed a comparable result, with 85.0% of cases demonstrating (partial) positivity in MIBG imaging at the tumour site. These findings indicate that MIBG treatment may be a viable therapeutic option for older patients as well, thus warranting further exploration and consideration in clinical practice.

Moreover, having received a completed sequence of immunotherapy was significantly associated with higher overall survival (p=0.008), but not with event-free survival (p=0.194). This is interesting, because challenge in treating high-risk neuroblastoma is the low immunogenicity of neuroblastoma tumours.(22) Previous research has also found an effect size of immunotherapy of approximately 20%.(23) Our current study reveals a substantially higher proportion, with a noteworthy 40% of patients demonstrating a positive response on survival to immunotherapeutic interventions. These findings indicate the possibility of a distinct treatment response pattern in this specific age group, indicating that immunotherapy may be particularly effective in older neuroblastoma patients.

Molecular

We have found a MYCN mutation in 16.6% of the cases. This is below the overall incidence, which is approximately 25%.(10) This affirms our hypothesis. Also, a recent study describes that ATRX and MYCN are not compatible.(24) In our population we only have 1 single patient who had a mutation of both. This strengthens the assumption of incompatibility. Furthermore, ATRX mutation has been described as more frequent among older neuroblastoma cases and having a poor prognosis.(8) Overall survival for ATRX mutation in our study was lower, but not significantly associated with survival. The incidence rate of this mutation was 15.2%. This is moderately lower than described in literature, viz. 20% for older children.(21, 25)

Moreover, the loss of heterozygosity 1p was close to significantly associated with overall survival and event-free survival. This corresponds to known literature.(26) The absence of a significant association could potentially be attributed to the limited population size.

The prevalence of ALK aberrations in neuroblastoma has been the subject of scientific investigation. While existing research indicates an ALK aberration rate of 9% among overall neuroblastoma cases, our own research has identified a higher incidence of 20%. This finding aligns with previous studies that have also reported a 20% prevalence of ALK aberrations in older neuroblastoma patients.(21)

The presence of gain of chromosome 17q, has been identified in more than half of neuroblastoma cases and is associated with a highly aggressive phenotype.(27, 28) Our study investigated the incidence rate of gain 17q and its potential impact on patient survival outcomes. Our findings revealed an incidence rate of 77.1% for gain 17q in the studied cohort. This appears to be higher than the overall rate of gain 17q. However, our analysis did not yield a significant association between the presence of gain 17q and patient survival.

Strengths and limitations

The present study had a limited sample size due to the inclusion criteria of the population group, which consists of children diagnosed with a rare form of cancer and are age selected, which has a lower prevalence. The rarity of this diagnosis inherently poses challenges in recruiting a large cohort for research purposes. However, despite the small sample size, we have found valuable insides. Further research may confirm our findings. It can be assumed that more data will be collected over time, because molecular tests will be done more often henceforth. Whole Exome Sequencing is now a standardized diagnostic test, and all neuroblastoma patients are treated in the Princess Maxima Center. This ensures that we will get a complete picture of this patient population, whereby our study can be a start of this overview.

Future research

This research initiative served as an initial exploration into the characterization of older neuroblastoma patients, providing a foundation for future investigations in this specific population. The findings obtained from this study offer valuable insights and pave the way for subsequent research endeavours, which can benefit from the advancements in technology and data availability that exist today. The current landscape of research possibilities presents an opportune environment for further studies to delve deeper into understanding the complexities of neuroblastoma in older patients.

We have found comparable MIBG positivity among our study population and known literature. Also, immunotherapy has demonstrated a significant improvement in survival

outcomes among patients. These findings could be a lead to novel therapeutic approaches. Identifying new therapeutic strategies will lead to improvement on current treatments and therefore better the prognosis of older neuroblastoma patients.

Additionally, there is a potential avenue for expanding research through the utilization of PALGA biopsies, a comprehensive pathology database that contains a wealth of patient information. Furthermore, the inclusion of international data from the International Neuroblastoma Risk Group (INRG) could contribute to an international approach of research data. The integration of PALGA biopsies and international data from INRG presents exciting prospects for future research endeavours, allowing for a more comprehensive understanding of older neuroblastoma patients. By adding these research opportunities, we can collectively advance the field and contribute to improved management and outcomes for this patient population.

Conclusion

We have made an overview of the characteristics of older neuroblastoma patients. The hypothesis that other clinical and molecular features play a role in older children is hereby supported. These findings suggest a potentially unfavourable prognosis for patients aged six years and above. Also, a higher number of high-risk patients has been found and molecular differences, like LOH1p, gain of 17q, more ALK mutations, less MYCN, seem to play a role in neuroblastoma patients 6 years and above. Additionally, immunotherapy had a substantially better survival and may be particularly effective in older neuroblastoma patients.

By investigating neuroblastoma in older children, this research contributes to the existing body of knowledge and provides valuable insights into the unique characteristics and complexities of the disease within this specific age cohort. This study initiates a comprehensive overview of the patient population, setting the stage for future research and novel therapeutic approaches to build upon.

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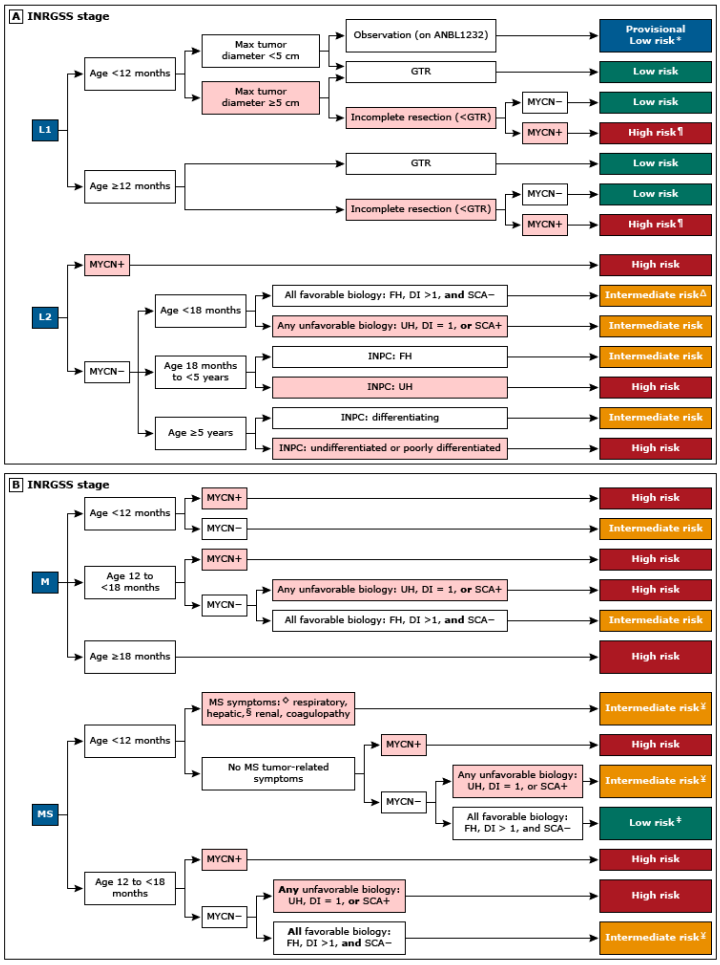
Appendix 1 INGRSS algorithm for classification.(14)

Algorithm for the COG Neuroblastoma Risk Classifier (version 2)



Risk classifier v2 algorithm for patients with (A) locoregional and (B) metastatic tumors. (A) Patients with locoregional tumors with neuroblastoma and ganglioneuroblastoma (nodular) are classified based on INRG stage (L1 and L2), age, resection, biomarkers (MYCN, ploidy, and SCAs), and INPC. Select patients with all favorable features are eligible for surveillance on the current non-high-risk COG ANBL1232 protocol. L1 or L2 tumors with histopathology diagnostic category of ganglioneuroma or ganglioneuroblastoma-intermixed will be classified as low risk regardless of biomarkers (and thus are not included in the figure). Ages are broken down by <18 months, 18 months to <5 years, and ≥5 years based on age categories used by INPC. (B) Patients with metastatic tumors are classified by stage (M and MS), age, INPC, and biomarkers. For MS patients, presence or absence of symptoms may influence therapy independent of biomarker status. In previous risk classifiers, missing data were considered as unfavorable. In COG v2, missing data for SCA will not be considered as unfavorable based on the low incidence of SCA in otherwise favorable subsets.

COG: Children's Oncology Group; GTR: gross-total resection; MYCN+: *MYCN* amplified; MYCN−: *MYCN* not amplified; FH: favorable histology; DI: DNA index; SCA: segmental chromosomal aberration; UH: unfavorable histology; INPC: International Neuroblastoma Pathology Classifier; v2: version 2; INRG: International Neuroblastoma Risk Group.

* If tumor progresses during observation, biopsy or resect and reclassify with biomarkers (as in COG ANBL1232).

¶ Consider complete resection if feasible.

Δ If no tumor burden symptoms, consider observation (as in COG ANBL1232).

◇ Biopsy contraindicated, defer biopsy until stable (NOTE: Biomarker results may modify risk class).

§ Hepatomegaly alone is an MS symptom in patients age <3 months of age (refer to COG ANBL1232).

¥ Response-based therapy (as in COG ANBL1232).

‡ MS score-based therapy (as in COG ANBL1232).

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Appendix 2 List of molecular characteristics.

MUTATION	WES	SNP ARRAY	NGS
	ABL	ALK	ABL1
	AKT1	MYCN	AKT1
	ALK	MDM2	ALK
	AMELY	CDK4	AMELY
	APC		APC
	ARAF		ARAF
	ATM		ATM
	BRAF		BRAF
	CALR		CALR
	CCND1		CCND1
	CDH1		CDH1
	CDK4		CDK4
	CDKN2A		CDKN2A
	CRAF1		CRAF (RAF1)
	CSF1R		CSF1R
	CTNNB1		CTNNB1
	DDX3Y		DDX3Y
	EGFR		EGFR
	ERBB2		ERBB2
	ERBB4		ERBB4
	EZH2		EZH2
	FBXW7		FBXW7
	FGFR1		FGFR1
	FGFR2		FGFR2
	FGFR3		FGFR3
	FLT3		FLT3
	GNA11		GNA11
	GNAQ		GNAQ
	GNAS		GNAS
	HNF1A		HNF1A
	HRAS		HRAS
	IDH1		IDH1
	IDH2		IDH2
	JAK2		JAK2
	JAK3		JAK3
	KDR		KDR
	KIT		KIT
	KRAS		KRAS
	MAP2K1		MAP2K1
	MDM2		MDM2
	MET		MET
	MLH1		MLH1
	MPL		MPL
	MYCN		MYCN
	MYD88		MYD88
	NOTCH1		NOTCH1
	NPM1		NPM1
	NRAS		NRAS
	PDGFRA		PDGFRA
	PIK3CA		PIK3CA
	POLD1		POLD1
	POLE		POLE
	PTEN		PTEN
	PTPN11		PTPN11
	RB1		RB1
	RET		RET
	ROS1		ROS1
	SMAD4		SMAD4
	SMARCB1		SMARCB1
	SMO		SMO
	SRC		SRC
	STK11		STK11
	TERT		TERT
	TP53		TP53
	VHL		VHL
	AMER1 (kidney)		AMER1 (kidney)
	FBXW7 (kidney)		FBXW7 (kidney)
	SMARCA4 (kidney)		SMARCA4 (kidney)
	SMARCB1 (kidney		SMARCB1 (kidney
	TP53 (kidney)		TP53 (kidney)
	VHL (kidney)		VHL (kidney)
	WT1 (kidney)		WT1 (kidney)
	WT1 (kidney)		WT1 (kidney)

Appendix 3 Additional figures.

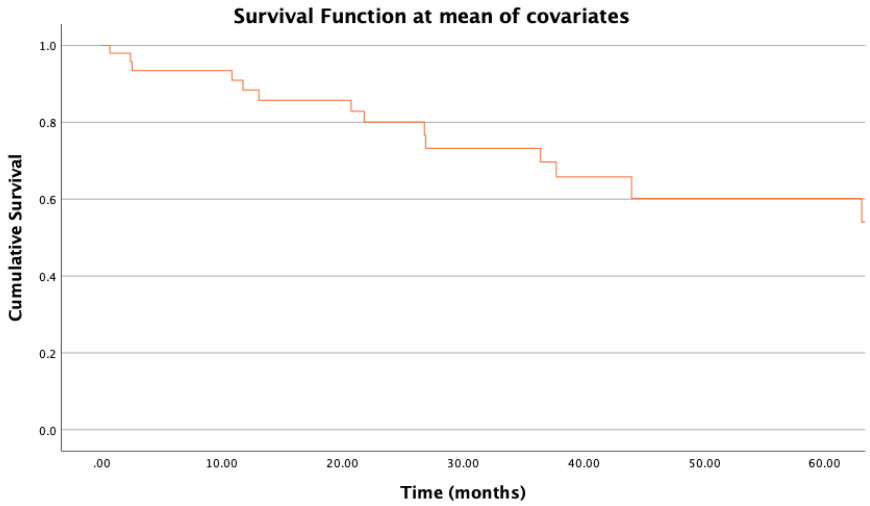

Figure 3. Survival function corrected for covariates LOH1p, ATRX and Risk groups. p=0.129

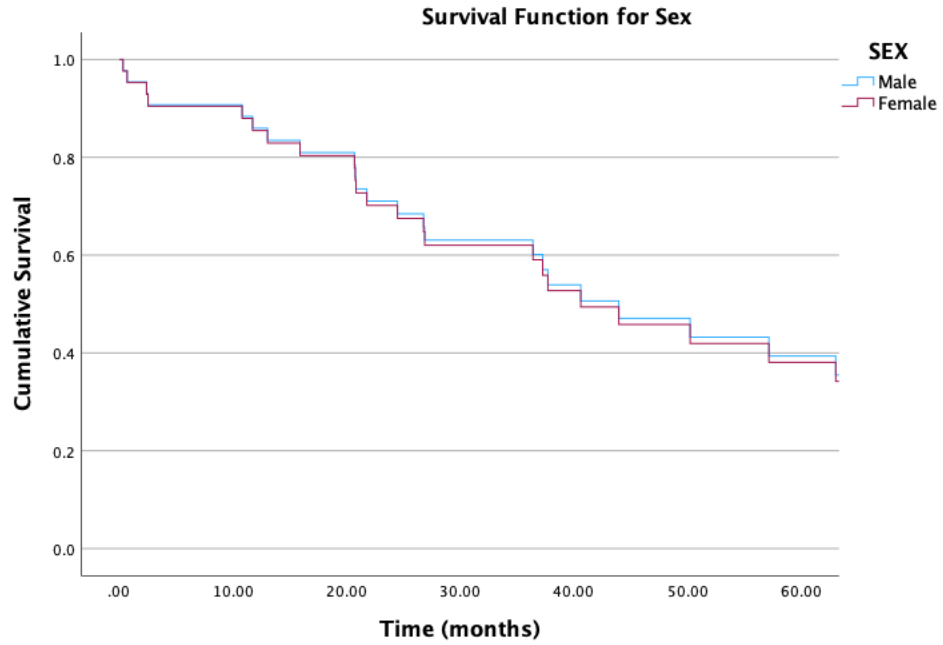

Figure 4. Survival function for Sex. (HR 0.982; 95% CI 0.654-1.475; p=0.931)

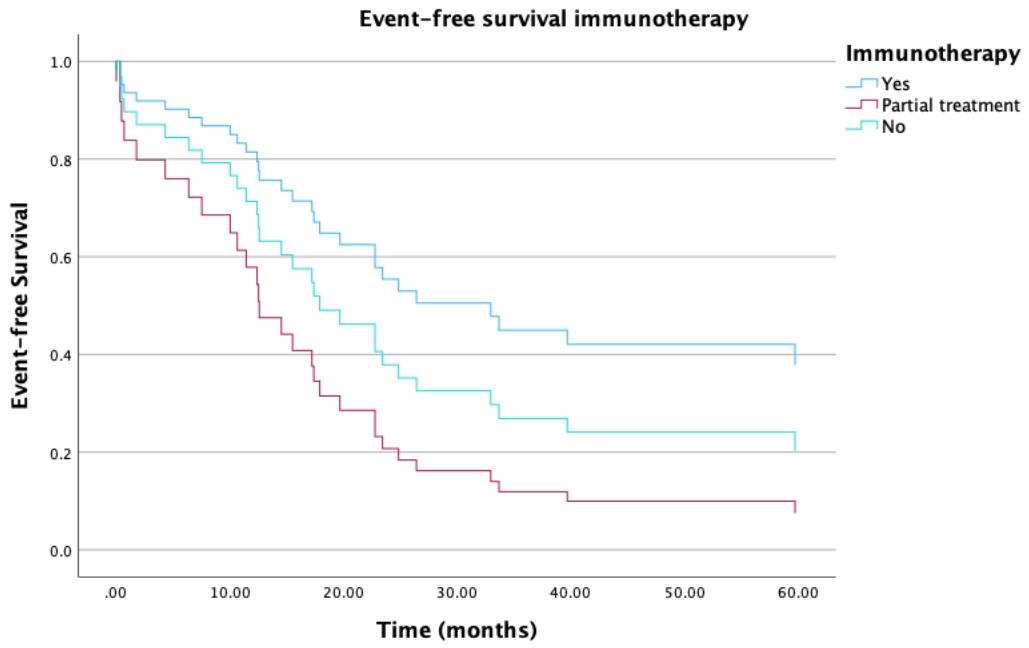

Figure 5. Event-free survival function for immunotherapy. (HR 2.665; 95% CI 0.888-7.999; p=0.194)

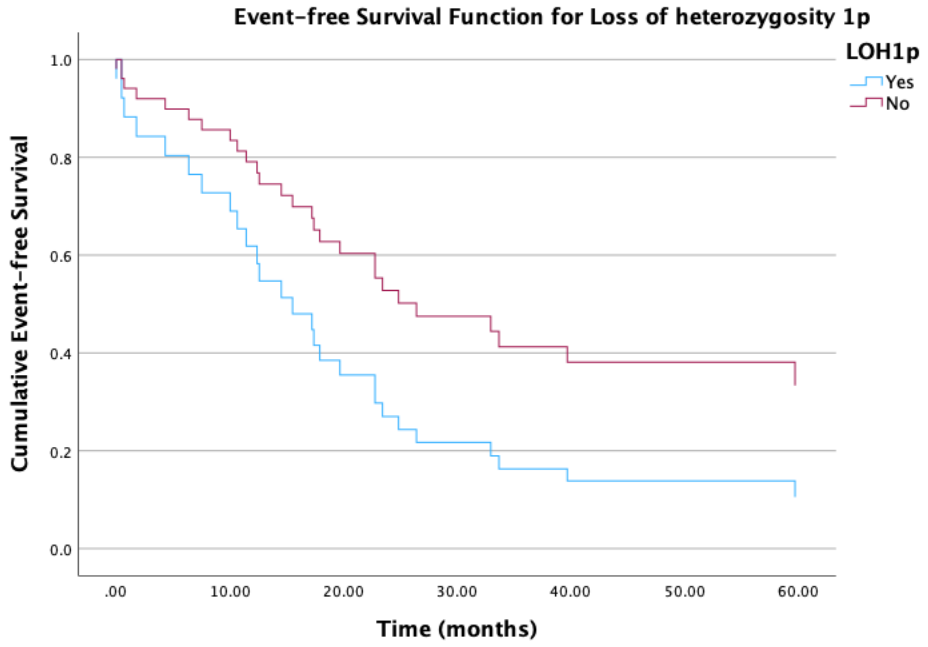

Figure 6. Event-free survival function Loss of heterozygosity 1p. (HR 0.488; 95% CI 0.216-1.103; p=0.085)

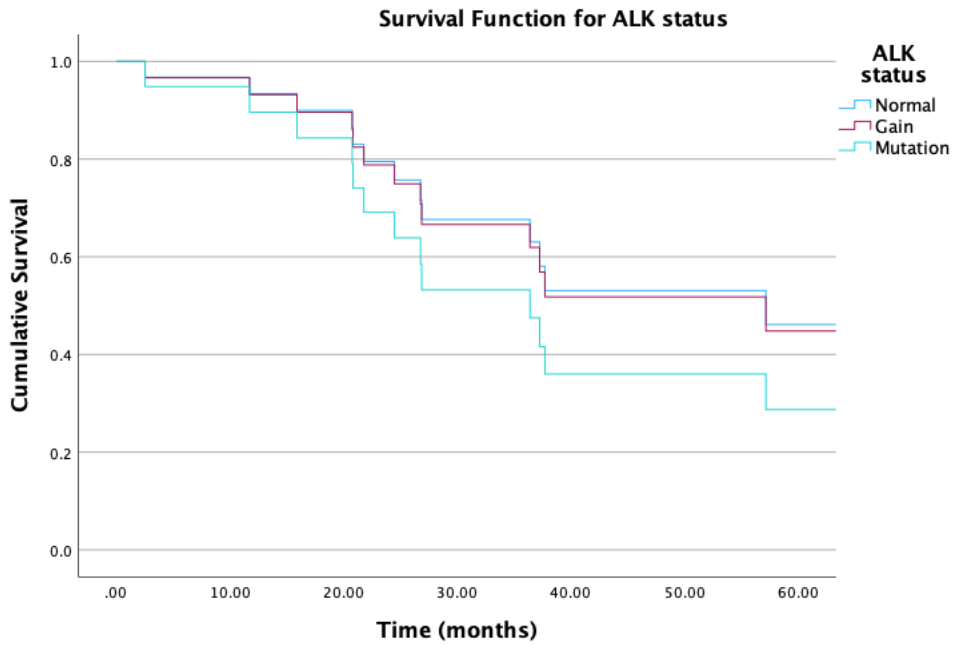

Figure 7. Survival function for ALK status (HR 1.227; 95% CI 0.586-2.566; p=0.588).

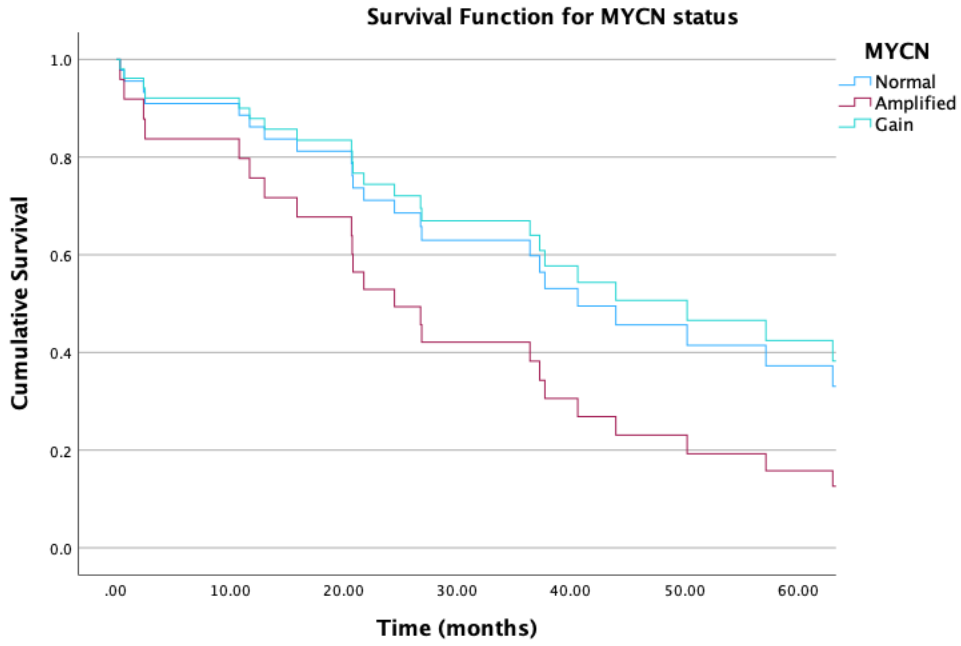

Figure 8. Survival function for MYCN status. (HR 1.018; 95% CI 0.534-1.940; p=0.957)