Characteristics and survival in children with high-risk neuroblastoma: A report from 38 Norwegian children treated according to the SIOPEN HR NBL-1 Study

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Project thesis – The Faculty of Medicine

UNIVERSITY OF OSLO

February 2018

Abstract

Background: Neuroblastoma is the most common extracranial solid malignant tumor of infancy, and it accounts for 15% of all pediatric oncology deaths. Clinical and biological factors in neuroblastoma have been studied for more than two decades, aiming at improving clinical outcome with risk-based treatment approaches. High-risk patients remain with overall survival rates less than 50% despite intensive multimodal therapy. Clinical protocols are still ongoing nowadays, collaborating internationally on evaluating new treatment strategies, with high-risk patients within Europe being encouraged to enroll on the High-Risk Neuroblastoma Study of the International Society for Pediatric Oncology-Europe (SIOPEN).

Material and Method: This is a descriptive longitudinal prospective study on Norwegian children diagnosed with high-risk neuroblastoma between April 2003 – December 2015, based on data registered in the database (SIOPEN-R-NET) created for the SIOPEN HR NBL-1 study. Patient characteristics at diagnosis and several changes observed during treatment for metastatic disease status and biochemical tumor markers were described and explored, as well as survival rates that were at last investigated in association to the prognostic factors of age and MYCN amplification.

Results: A total of 38 children were included, with a predominance of males and a median age of three years and three months. Most children had INSS tumor stage 4. Almost all of the children had elevated biochemical tumor markers at diagnosis, which disappeared during treatment in accordance with remission status. Results for survival were better than expected, with an overall 5-year survival rate of 74%. We were not able to find any significant association for survival outcome with age or MYCN amplification.

Conclusion: Norwegian children with high-risk neuroblastoma should continue to be enrolled on the SIOPEN study as international collaboration is essential, with new treatment strategies to be designed for subgroups of patients. It would be of clinical interest to evaluate the cause of death, acute and chronic complications from treatment, and quality of life for long terms survivors in further analyses.

Abstrakt

Bakgrunn: Nevroblastom er den vanligste ekstrakranielle solide ondartede svulsttypen hos små barn, og står for 15% av alle pediatriske onkologiske dødsfall. I over 20 år har kliniske og biologiske faktorer vært studert for å optimalisere stratifisert behandling og prognose for barn med nevroblastom. Til tross for intensiv multimodal behandling er langtidsoverlevelsen for høyrisiko pasienter under 50%. Det samarbeides gjennom internasjonale kliniske protokoller for å evaluere nye behandlingsstrategier, hvor barnekreftsentra oppfordres til å inkludere pasienter med nevroblastom i behandlingsprotokoller i regi av den europeiske nevroblastom organisasjonen (SIOPEN); en gren av den internasjonale barnekreft organisasjonen SIOP (International Society for Pediatric Oncology).

Materiale og metode: Dette er en deskriptiv longitudinell prospektiv studie av norske barn diagnostisert med høyrisiko nevroblastom mellom april 2003 og desember 2015, basert på data registrert i databasen (SIOPEN-R-NET) laget for SIOPEN HR NBL-1 studien. Pasientkarakteristika ved diagnose og noen endringer i metastatisk sykdomsstatus og biokjemiske tumormarkører har blitt studert, i tillegg til overlevelse med overlevelsesanalyser for alder og MYCN amplifikasjon som prognostiske faktorer.

Resultater: Totalt ble 38 barn inkludert, med en større andel av gutter og en medianalder i hele gruppen på tre år og tre måneder. De fleste av barna hadde INSS tumorstadium 4. Nesten alle av barna hadde forhøyete biokjemiske tumormarkører ved diagnose, som normaliserte seg i løpet av behandling i henhold til remisjonsstatus. Observert overlevelse ble bedre enn forventet, med en totaloverlevelse ved 5 år på 74%. Vi fant ingen signifikant forskjell i overlevelse i forhold til alder eller MYCN amplifikasjon.

Konklusjon: Norske barn med høyrisiko nevroblastom bør behandles i internasjonale studieprotokoller, for eksempel SIOPEN-protokoller, siden internasjonalt samarbeid er avgjørende for å utvikle kunnskapsbaserte behandlingsstrategier for alle undergrupper av pasienter. Videre vil det være av klinisk interesse å utforske dødsårsak, akutte komplikasjoner og langtidskomplikasjoner av behandling og livskvalitet hos de overlevende i utvidede analyser.

Abbreviations

Abbreviations and descriptions used in the HR NBL1.7/SIOPEN study protocol:

CR: Complete Remission – no tumor, catecholamines normal

VGPR: Very Good Partial Remission – decreased by 90-99%, catecholamines normal, residual technetium scan changes allowed (MIBG negative at all metastatic sites)

PR: Partial Remission – decreased by > 50%, All measureable sites decreased by > 50%. Bones and bone marrow: number of positive bone sites decreased by > 50%, no more than 1 positive bone marrow site allowed (one positive marrow aspirate or biopsy allowed if this represents a decrease from the number of positive sites at diagnosis)

Minor Response – 50% reduction / < 25% increase, no new lesions, > 50% reduction in measurable lesion (primary or metastases) with < 50% reduction in any other, < 25% increase in any existing lesion

SD: Stable Disease – 50% reduction/ < 25% increase, no new lesions, < 50% reduction but < 25% increase in any existing lesion

PD: Progressive Disease – increase > 25%, any new lesion, increase of any measurable lesion by > 25%, previous negative marrow positive for tumor

NE: Not Evaluable

Contents

1 Introduction

Childhood cancer is a rare disease (it accounts for less than 1% of all cancers in industrialized countries), yet it is sadly enough the most common cause of death from disease in children and adolescents [1]. Neuroblastoma is the most common extracranial solid pediatric malignant tumor, typically affecting children younger than five years old. It accounts for 15% of cancer-related deaths in children. The clinical heterogeneous phenotype is a hallmark of neuroblastoma and its prognosis ranges from spontaneous regression in neonates to aggressive metastatic tumors in older children [2].

It is for more than two decades that neuroblastoma treatment has served as a paradigm for the incorporation of clinical and biological factors to stratify patients and tailor therapies. Age, tumor stage, histopathology, and tumor genetics are identified to be the most robust prognostic factors. Outcomes for low- and intermediate-risk neuroblastoma patients are considered to be good, while long-term survival of high-risk patients, despite intensification of treatments and incorporation of immunotherapies, remain less than 50% [3].

The case of high-risk neuroblastoma patients undoubtedly remains a great challenge for pediatric oncologists. The vast amount of clinical and biological information collected in the last 20 years suggests it is time to turn to more personalized therapies. There are many unresolved issues on the case and ongoing clinical protocols collaborate internationally on evaluating new treatment strategies for targeting patients. High-risk neuroblastoma patients are encouraged to enroll on study protocols such as the High-Risk Neuroblastoma Study of SIOPEN.

This paper aims at describing and exploring high-risk neuroblastoma patients in Norway treated according to the SIOPEN HR NBL-1 protocol. A main focus has been put on the prognostic factors of age at diagnosis and the amplification of the MYCN oncogene in relation to the survival outcome of the study population. Analysis of data registered in the study database (SIOPEN-R-NET) of the affected children in Norway between April 2003 and December 2015 allows for this paper a descriptive longitudinal prospective study.

2 Background information

2.1 Cancer in children

Approximately 200 children and adolescents (under 18 years of age) get diagnosed with cancer in Norway every year [1]. Cancer in children is rare, the etiology is in most cases unclear, and the disease differs itself in many ways from cancer in adults. There are many forms of cancer in children and they exhibit a great histological diversity, with some types of tumors arising in many different primary loci. Childhood cancers are in fact classified according to histology [4]. Malignancy in children might clinically present as a localized mass or its pressure effects or the consequences of disseminated disease [5].

Cancer in children is classified into diagnostic groups: leukemia, lymphoma, central nervous system neoplasms, neuroblastoma and other peripheral nerve-cell tumors, retinoblastoma, renal tumors, hepatic tumors, bone sarcomas, soft-tissue sarcomas, germ-cell neoplasms, and others. These diagnostic groups could be parted into three major groups: one-third representing leukemia, one-third representing central nervous system tumors, and the last third representing solid tumors; lymphoma being the most common one in this latter group [1]. Leukemias are the most common childhood cancers, affecting children at all ages (although there is an early childhood peak). Neuroblastoma is most commonly seen in children under five years, and Wilms tumor is almost always seen in the first six years of life. Hodgkin lymphoma and bone tumors have their peak incidence in adolescence and early adult life [5].

The prognosis for cancer is generally speaking better for children compared to adults. Children are however in continuous growth with an increased risk of acute and long-term toxicity. Multidisciplinary collaboration is essential in pediatric oncology. Treatment might involve chemotherapy, surgery, radiotherapy, or high-dose therapy with stem cell rescue [1].

In Norway, treatment of childhood cancer is centralized to the University Hospitals in each of the four health regions (South-East, Middle, West and North). Each region has multidisciplinary teams who are responsible for the diagnostic work-up and treatment of the children. Random and natural differences in the occurrence of cancer in children from one year to another, together with the fact that there are few cases each year, leads to challenges for national registers in a country with a relatively small population like Norway. International collaboration is of great importance as changes in for example survival over time will often not be statistically significant before after many years [1].

2.2 The case of neuroblastoma

Neuroblastoma accounts for 8-10% of all childhood cancers. Metastatic neuroblastoma in children over the age of one year is associated with the worst prognosis of all malignancies in childhood. Neuroblastoma primarily affects patients younger than five years of age with a median age at diagnosis of 19 months. Around 37% are diagnosed as infants, 50% are below the age of two years and 90% are younger than five years at diagnosis. The etiology of neuroblastoma is unclear but factors such as this early age of onset suggest that preconceptual or gestational events might play a role [6].

Age, one of the first prognostic factors identified, is still said to be a paramount factor in determining the prognosis for neuroblastoma patients. Through analysis, a cut-off age of around 18 months has been suggested for the optimal prognostic distinction. Patients less than 18 months of age are said to have better outcome, even with bone marrow involvement [6]. It should at this point also be mentioned that five years appears to be the next cut-off age for worsening prognosis because children over five years at the time of diagnosis seem to have very poor long-term prognosis, with a survival rate less than 20% (unpublished SIOPEN data).

Neuroblastoma originates from primitive neural crest, arising at any location coinciding with normal sympathetic ganglia. The adrenal gland is the most common primary site (40%), followed by other abdominal (25%), thoracic (15%), cervical (5%) and pelvic sympathetic ganglia locations. The most common clinical presentation is abdominal mass. An infant may present with a grossly enlarged liver with subcutaneous nodules. Cervical and thoracic tumors are more common in infants which may have the Horner syndrome and respiratory symptoms [6, 7].

Approximately half of patients with neuroblastoma have localized or regional disease, and 35% have regional lymph node spread at the time of diagnosis. Distant metastatic disease is seen in 50% of patients at diagnosis and occur through lymphatic and hematogenous routes. Bone, bone marrow and liver are the most common metastatic sites. Neuroblastoma has a particular predilection to spread to metaphyseal, skull and orbital bone sites, resulting in a classic presentation characterized by periorbital ecchymosis (“raccoon eyes”), proptosis and potential visual impairment [6].

2.3 Gene aberrations

In neuroblastoma, a number of genetic aberrations have been identified to be strongly related to clinical outcome. Amplification of the MYCN oncogene is observed in about 20% of tumors and is clearly associated with a poor outcome. MYCN amplification is in fact seen to give a rapid progression of disease in subgroups of patients and could be said to remain the more reliable prognostic marker among genes that have been found abnormal in neuroblastoma. It however needs to be stated that the majority of metastatic aggressive tumors do not show MYCN amplification, indicating the way other genes have an important role in tumor aggressiveness [8].

There are a number of segmental chromosomal alterations (SCA) reported for neuroblastoma, including deletion of chromosomes 1p, 3p, 4p and 11q, as well as gains of chromosomes 1p, 2p and 17q. Recent analysis suggests that 17q gain, 1p deletion and 11q deletion are the most frequent segmental chromosome alterations in tumors without MYCN amplification. Allelic loss of 11q is present in 35-45% of primary tumors and is seen to be highly associated with high-risk features. A gain of 1-3 additional 17q copies, often through unbalanced translocation with chromosome 1 or 11, is also said to correlate with a more aggressive phenotype [2, 9].

2.4 Histopathology

Neuroblastoma is microscopically characterized by uniform small round blue cells with hyperchromatic nuclei with varying degree of differentiation [11].
The International Neuroblastoma Pathology Classification System (INPC) was established in 1999 and is a modification, made in 2003, of the classification scheme proposed by Shimada et al in 1984. The tumors get classified as histologically favorable or unfavorable depending on the degree of neuroblast differentiation, Schwannian stroma content, mitosis karyorrhexis index and age at diagnosis [12]. The undifferentiated stroma-poor neuroblastoma is the most aggressive tumor, in particular when occurring in children over the age of 12 months [13]. It is suggested that the incorporation of age in both the Shimada system and INPC system makes age-linked histopathological classification serve better than age by itself for prognostic distinction of neuroblastoma patients in infants and younger children [12].

Furthermore, unfavorable histology is said to have a strong association with amplification of the MYCN oncogene. It has in fact been observed that tumors with MYCN amplification are

usually undifferentiated or poorly differentiated subtypes of neuroblastoma (Schwannian stroma poor), with markedly increased mitotic and apoptotic activities [14, 15].

2.5 Diagnosis

The diagnosis of neuroblastoma is confirmed either by tumor tissue biopsy and histopathology or by a combination of neuroblastoma tumor cells detected in bone marrow aspirates, along with elevated urine or serum catecholamines (dopamine, vanillylmandelic acid and homovanillic acid) [16].

Children presenting with suspicion of neuroblastoma are evaluated by cross-sectional imagining (CT scan or MRI) in order to assess the extent of primary tumor and to evaluate the regional or distant spread of disease [2].

Meta-iodobenzylguanidine (MIBG) is taken up preferentially into cells of the sympathetic nervous system involved in catecholamine synthesis. If the compound is radiolabeled it can localize primary and metastatic neuroblastomas, with high specificity. MIBG scan is thus utilized to detect metastatic disease and to monitor patients throughout the various treatment phases. However, not all primary tumors or metastatic disease is MIBG-avid, and patients that have MIBG-negative tumors should always get a technetium bone scan preformed to evaluate cortical bone involvement [17].

Metastatic disease in bone marrow is one of the most notable occurrences in poor prognosis for patients with neuroblastoma. The consensus internationally is that all patients should have core biopsies and bilateral posterior iliac crest aspirates of the bone marrow carried out before bone marrow involvement would be possible to exclude in patients [2, 16].

Neuroblastoma is a biologically active tumor that secretes catecholamines in the plasma and urine. Measuring the levels of the catecholamines vanillyl mandelic acid (VMA) and homovanillic acid (HVA) in urine has been proven to be useful in the initial diagnostic and follow-up evaluation of children with neuroblastoma. Elevated levels of lactate dehydrogenase (LDH), serum ferritin and neuron-specific enolase (NSE) may also be useful for diagnostic purposes and for the prognostication of patients, however these factors lack sensitivity and specificity in monitoring the disease activity [6,17].

2.6 Staging

Standard staging criterias of neuroblastoma has classically been based on the International Neuroblastoma Staging System (INSS). INSS has been in practice since 1988 and is assigned after surgical resection and relies on the pathologic assessment of resected tumors and lymph nodes [2, 17]. A newer presurgical staging system, the International Neuroblastoma Risk Group Staging System (INRGSS) has been developed using image-defined risk factors (IDRF) for pretreatment risk stratification [18].

International Neuroblastoma Staging System (INSS)

Stage 1: Localized tumor confined to the area of origin; complete gross excision with negative lymph nodes
Stage 2: Localized tumor with incomplete gross excision or positive ipsilateral lymph node
Stage 3: Tumor extending across the midline or with bilateral lymph node involvement
Stage 4: Dissemination of tumor to distant lymph nodes, bone, bone marrow, liver or other organs
Stage 4S: Localized primary tumor (like in stage 1 and 2) in children < 1 year old with metastasis to liver, skin and/or bone marrow (skeleton NOT affected)

International Neuroblastoma Risk Group Staging System (INRGSS)

Stage L1: Localized tumor not involving vital structures as defined by image-defined risk factors (IDRFs) and confined to one body compartment
Stage L2: Locoregional tumor with presence of one or more IDRFs
Stage M: Distant metastatic disease
Stage MS: Metastatic disease in children younger than 18 months with metastasis confined to skin, liver and/or bone marrow

2.7 Treatment and clinical outcome

The treatment varies among the risk categories (low, intermediate and high) from “wait and see” or only surgery, to highly intensive protocols with all cancer therapy modalities taken into use [19]. For patients with low risk tumors, surgery alone, low dose chemotherapy or observation is the primary “treatment”. Patients who relapse within this category can generally be salvaged with further surgery or chemotherapy [20]. Among patients with more advanced

neuroblastoma (intermediate or high-risk, depending upon other prognostic factors), treatment and outcome varies according to gene aberrations, age at diagnosis and histologic features [3].

Patients with high risk neuroblastoma include children older than 12-18 months of age with disseminated disease at diagnosis, or children at any age with disseminated or localized disease with unfavorable biology (MYCN amplification). Patients do poorly even though treatment for this category of patients involves intensive multimodality strategies with autologous hematopoietic stem cell rescue. The long-term survival rates are approximately 40%. Standard treatment consists of induction chemotherapy and local control, consolidation therapy with high-dose chemotherapy and post-consolidation therapy with immunotherapy [3, 19].

3 Aim

The aim of this paper is to describe Norwegian children with high-risk neuroblastoma, treated according to the SIOPEN HR NBL-1 protocol. Data on patient characteristics at diagnosis, course of disease and survival are described and explored. A main focus has been put on the prognostic factors of age at diagnosis and MYCN amplification for the group of patients studied as a whole. In the last part of this present study, the prognostic factors mentioned are put in relation to the survival outcome of the group of children analyzed.

4 Material

Patients included in this study were children in Norway diagnosed with high-risk neuroblastoma from April 2003 to December 2015 (enrolled and treated according to the SIOPEN HR NBL-1 protocol), resulting in a total number of 38 children. The patients were treated at University Hospitals from each of the four health regions in Norway. Nineteen of the patients were from Rikshospitalet (Oslo University Hospital), nine patients were from Haukeland University Hospital, five patients were from University Hospital of North-Norway, and five patients were from St. Olavs Hospital Trondheim. Children diagnosed and enrolled in the SIOPEN study at later dates than in December 2015 were not selected as this project thesis is time-limited, allowing to only make observations from data records covering a limited period of time. A minimum of two years to follow-up was assured for those patients included in this study.

5 Method

5.1 Patient data

All data was collected from data registered in the study database (Web-based SIOPEN Research Network: SIOPEN-R-NET; https://www.siopen-r-net.org) of the randomized study of European SIOP Neuroblastoma (SIOPEN) Group in high-risk neuroblastoma (stage 2, 3, 4 and 4S MYCN-amplified neuroblastoma, stage 4 MYCN non-amplified neuroblastoma).

As data was collected from the database, patient data was organized into tables in Microsoft Word and Excel, in which all patients were assigned a distinct number (for anonymization), consistently representing each one of the children all throughout the study.
No data registered in the study database or previous reporting not corresponding with current descriptions used in the HR-NBL 1.7/SIOPEN study protocol was considered as "no data" and thus not included in observations made in this study. Due to limited access to patient records (the database includes data from multiple hospital centers), data was not further checked in patient records if missing from the database. Some variables resulted in having data missing for most of the patients and were therefore not included in analysis and observations made in this study.

5.2 The study protocol

The protocol of HR-NBL 1.7/SIOPEN study consists of a rapid, dose intensive induction chemotherapy, peripheral blood stem cell harvest, attempted complete excision of the primary tumor, myeloablative treatment (BUMEL MAT) followed by peripheral blood stem cell rescue, radiotherapy to the site of the primary tumor, and at last the MRD-phase consisting of Vitamin A with or without immunotherapy (see appendix I).

Induction therapy aims at clearing the patient's body of disease as much as possible particularly from distant sites, often bone and bone marrow. For induction, all of the 38 patients received Rapid COJEC, consisting of eight courses of chemotherapy every ten days. Rapid COJEC was from November 2005 given together with G-CSF support for all patients (based on the results of the R0 randomisation running between 2002 and 2005) [21]. Six of the 38 patients were R0 randomised (three patients received G-CSF and three patients received no G-CSF).

Following induction therapy, peripheral blood stem cell harvest (PBSCH) was performed and complete excision of the primary tumor was attempted. Patients with an inadequate metastatic response to allow myeloablative treatment followed by peripheral blood stem cell reinfusion (PBSCR) at the end of induction were given two TVD (Topotecan, Vincristine, Doxorubicin) cycles.

BUMEL MAT (myeloablative treatment) has since October 2010 been the standard MAT of the protocol (following the results of R1 randomisation running between 2002 and 2010) [22]. Twelve of the patients were R1 randomised (seven of the patients received CEM and five of

the patients received BUMEL). CEM consisted of continuous infusion of carboplatin, etoposide and melphalan. The BUMEL MAT regimen consists of oral or intravenous administration of busulfan as a two-hour infusion every six hours over four or five consecutive days, as well as the short intravenous infusion of melphalan.

Following MAT, peripheral blood stem cells were reinfused via a central venous line and radiotherapy was given to the site of the primary tumor.

During MRD-treatment phase, all patients (apart from a few not alive by this phase) received isotretinoin and some were randomized (R2/R4) to immunotherapy with isotretinoin (13-cis-RA) and ch14.18/CHO, with or without aldesleukin (IL-2). Thirteen of the patients were R2/R4 randomized (nine patients received RA + Anti GD2 and four patients received RA + Anti GD2 + IL2). The patients received the anti-neuroblastoma monoclonal antibody (ch14.18/CHO) for five courses. For differentiation treatment, the patients received six cycles of isotretinoin, given by mouth divided into two equal doses over 14 days every four weeks.

5.3 Data variable analysis

Patient characteristics at the time of diagnosis were looked at for the children in this study through analyzing the following data variables: age, gender, tumor stage, primary tumor location, histopathology, MYCN amplification, metastasis and tumor markers. The classical International Neuroblastoma Staging System (INSS) is the staging criteria used in the reporting of tumor stage in this study as this is with what is used in the database. Tumor histopathology has been assessed according to the International Neuroblastoma Pathology Classification (INPC) system. Fluorescence *in situ* hybridization (FISH) has been utilized to identify MYCN amplification and the study protocol describes MYCN amplification as over 4-fold increase of the MYCN signal number in relation to the number of chromosomes 2. Tumor marker values from blood and urine analysis were considered “elevated” in this study if they were registered as “elevated” in the study database, thus not by using reference values.

The course of disease for the 38 children has been looked at through analyzing data from evaluations made after induction therapy, after myeloablative treatment and after MRD-treatment. Changes from one of these clinical phases to the other in evaluations made on metastatic disease in skeleton and bone marrow, as well as evaluations made on biochemical tumor markers, have been analyzed and put in a longitudinal perspective.

5.4 Statistical analysis

For the most part of this paper, descriptive statistics has been used as this present study did generally not have enough power required for analytical statistical analysis. Microsoft Excel has been used for numbers, tables and figures, as well as for range, median and mean values. Age at diagnosis was manually calculated in Excel from the date of birth and the date of diagnosis given in the database.

Survival analyses were performed using the methods of Kaplan and Meier with SPSS. Survival was calculated from the date of diagnosis to the last follow-up date (set at 31.12.2017 for those patients that were still alive) or to the date of death, assuming that all deaths were reported in the study database. For overall survival (OS), death from any cause was the only endpoint event. To investigate the association of survival to the prognostic factors of age at diagnosis and MYCN amplification, Kaplan-Meier survival curves were compared for each of the factors mentioned using Chi-square and log-rank test. P-values < 0.05 were considered statistically significant. [23]

5.5 Research ethics

The SIOPEN HR-NBL study is approved by Regional Committees for Medical and Health Research Ethics (REK) and by the Norwegian Medicines Agency (SLV). The REK number is 2010/266 S-01280 and the SLV (EudraCT) number is 2006-001489-17.
Informed consent is given for inclusion and registration. All data taken from the study database (SIOPEN-R-NET) was unidentifiable and data was anonymized while being collected and analyzed, as a quality research study. No personal data is given. No changes or adjustments have been made for the purposes of this study.

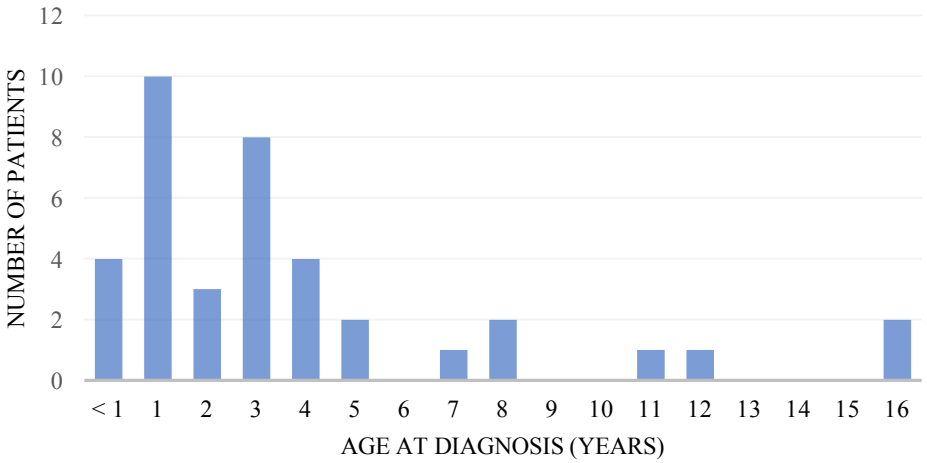
6 Results

6.1 Part 1: Patient characteristics at diagnosis

1 a) Age and gender

Age: The median age at which the 38 patients were diagnosed was three year and three months, ranging from one month to 16 years. The figure below illustrates a typical trend of neuroblastoma as most patients were younger than five years old at diagnosis (ten of these patients were aged under 18 months). Four patients distinguished themselves by being over ten years of age.

Figure 1: Age distribution at diagnosis



Gender: 22 (58%) of the patients were male and 16 (42%) were female.

1 b) Tumor data

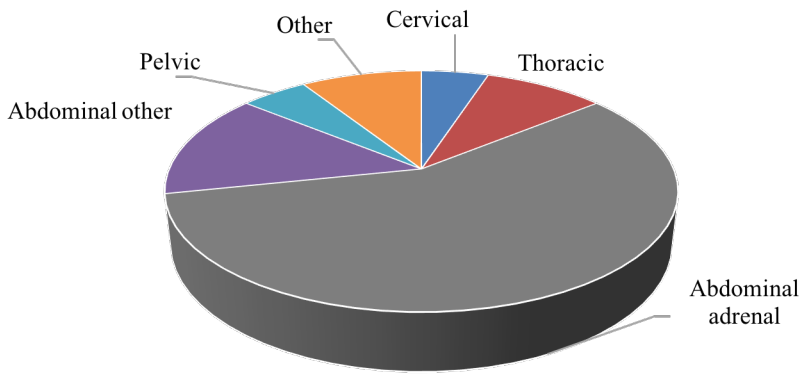
Tumor stage (INSS)

One patient had stage 2/3 disease, one patient had stage 3 disease, one patient had stage 4S disease, and 35 patients had stage 4 disease.

Primary tumor location

The primary tumor was located to only one region for 28 of the 38 patients. Most patients had their primary tumor located to the abdominal adrenal glands, as illustrated in figure 2.

Figure 2: Distribution of primary tumor site



Histopathology

The table below shows that all patients with data registered in the study database for tumor histopathology had their tumors classified as histologically unfavorable (undifferentiated or poorly differentiated subtypes of neuroblastoma).

Table 1: Tumor histopathology (INPC)

Tumor histopathology	Number of patients
Undifferentiated	3
Poorly differentiated	30
Differentiated	0
No data	5
Total	38

MYCN amplification

The number of patients without MYCN amplification almost balances up to the number of patients with MYCN amplification.

Table 2: MYCN Study (FISH)

MYCN amplification	Number of patients
Yes	17
No	20
No data	1
Total	38

1 c) Metastasis at diagnosis

The table below shows that the most common metastatic sites were skeleton and bone marrow, as expected. Only two patients had no metastatic disease at the time of diagnosis.

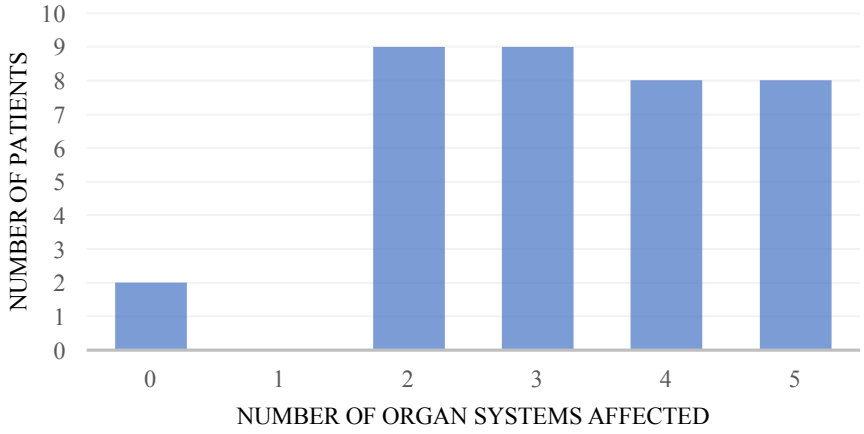
Table 3: Metastatic disease at diagnosis

Metastasis	Yes	No	No data
Skeleton*	29	9	0
Bone marrow	30	8	0
Liver	8	28	2
Lung	1	35	2
Lymphatic system	21	15	2

**For those patients that had a negative MIBG scan, the result registered in the database for the technetium bone scan preformed at diagnosis was here checked and considered, as not all metastatic disease is MIBG-avid and patients that have MIBG-negative tumors should get a technetium bone scan taken for further evaluation.*

The most common combination of organs/organ systems being affected at diagnosis was skeleton and bone marrow, with also metastasis in soft tissue and/or the lymphatic system for those patients with more than two organs/organ systems being affected.

Figure 3: Number of organ systems affected at diagnosis



The organ systems considered were the ones registered for in the study database: skeleton, bone marrow, soft tissue, liver, lungs, lymphatic system and "other organs and body parts".
Data was missing for 2 of the 38 patients.

1 d) Tumor markers at diagnosis

All the tumor markers (except ferritin) were elevated for most of the 38 patients at diagnosis.

Table 4: Blood and urine analysis

Tumor marker*	Yes	No	No data
Elevated LDH	32	4	2
Elevated Ferritin	13	19	6
Elevated NSE	33	1	4
Elevated VMA	24	8	6
Elevated HVA	29	2	7

**The tumor markers considered from the study database: LDH (lactate dehydrogenase), Ferritin, NSE (neuron-specific enolase), VMA (vanillyl mandelic acid) and HVA (homovanillic acid).*

Tumor markers were extremely high for a few patients, influencing the mean of the corresponding tumor marker. The median values in table 5 give a better indication of the typical tumor marker values for the 38 patients at diagnosis.

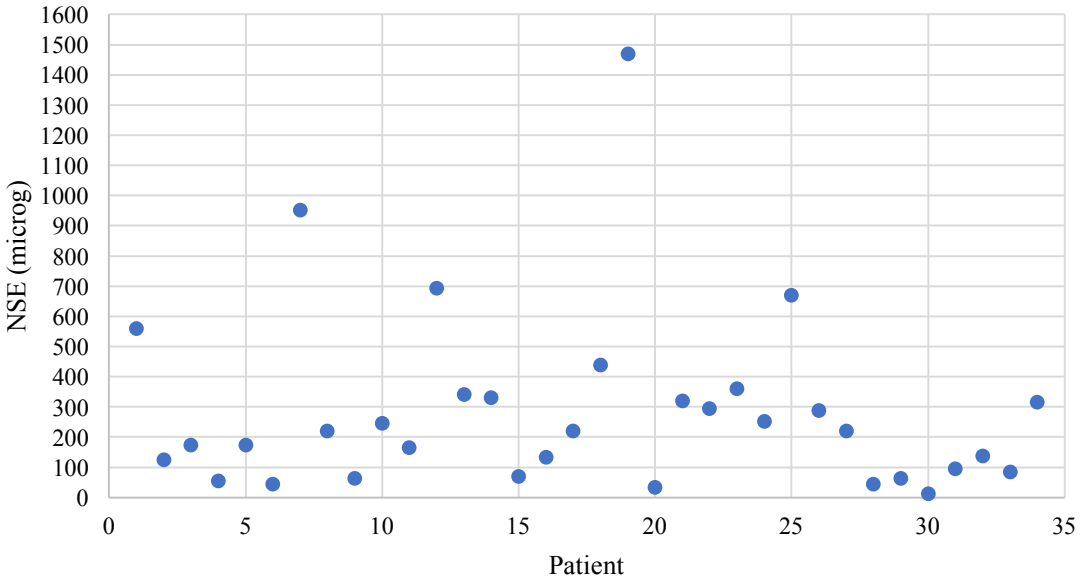
Table 5: Laboratory results at diagnosis

Tumor marker	Reference value*	Patient range	Patient median value	Patient mean value
LDH (IU/I)	≤ 430	139 – 5212	642	1114
Ferritin (microg)	≤ 140	38 – 792	186	222
NSE (microg)	≤ 12	12 – 1469	220	284
VMA (µmol/mmol creat)	≤ 20	2 – 460	67	98
HVA (µmol/mmol creat)	≤ 20	2 – 440	67	103

**The normal values for LDH, VMA and HVA vary according to the age of the child. The normal range of values for NSE has changed over time, and for this present study it was set at ≤ 12 micrograms.*
The patients that had no data registered in the study database were excluded from the calculations made for this table.

The distribution of values for NSE (neuron-specific enolase) at diagnosis is illustrated below for each of the patients that had their NSE values registered in the database (n = 34).

Figure 4: Distribution of NSE values at diagnosis



Summary of patient characteristics at diagnosis:

Most of the 38 patients were younger than five years at diagnosis, with a median age of three years and three months. There was a slight greater number of males than females. Most patients had INSS tumor stage 4. The adrenal gland was the most common primary tumor site. MYCN status was known for 37 patients, with amplification present in 17 patients. All patients that there was data for had unfavorable histology (undifferentiated or poorly differentiated), according to INPC. Only two patients presented with no metastatic disease, while for the rest of patients the most common metastatic sites were bone marrow and skeleton. Most of the 38 patients had elevated urine catecholamines, LDH and NSE at diagnosis.

6.2 Part 2: Course of disease

2 a) Evaluation of metastatic disease at various clinical phases

Evaluation of metastasis in skeleton

Table 6: MIBG Scintigraphy (Bone)

Metastasis in bone*	Post induction	Post MAT	Post MRD
CR	15	25	25
VGPR	0	0	0
PR	16	0	0
Minor Response	0	0	0
SD	5	1	1
PD	0	2	2
NE	0	0	0
Off trial	0	3	3
Not alive	1	2	2
No data	1	5	5
Total	38	38	38

**As a reference from the previous section it should be noted that 29 of the 38 patients were positive for metastatic disease in bone (MIBG scanning) at the time of diagnosis.*

Even though not following the single patient from one clinical phase to the other, table 6 sets the overall picture for changes in bone metastasis throughout treatment for the 38 children. There is a notable improvement from the time of diagnosis to the end of induction, with 15 patients in complete remission (CR). By the end of induction therapy, 16 patients were in partial remission (PR) and five patients in stable disease (SD). From this, improvements are seen in ten additional patients achieving complete remission by the end of myeloablative treatment (MAT). Three patients were though still in stable disease (SD) or progressive disease (PD) by the end of MAT, and the state of these patients in relation to their state by the end of induction is not clear to tell from table 6, also with patients going off trial and death always being a possible outcome. There are no changes observed between the end of MAT and the end of MRD-treatment.

Evaluation of metastasis in bone marrow

Table 7 gives an overview of bone marrow involvement during treatment. The greatest improvement in most patients resulting negative for bone marrow involvement is seen from diagnosis to the end of induction. Improvement is also noticeable by the end of MAT. No change is observed from the end of MAT to the end of MRD-treatment.

Table 7: Bone Marrow

Bone Marrow	At diagnosis	Post induction	Post MAT	Post MRD
Positive	**30**	**11**	**3**	**3**
Negative	**8**	**22**	**26**	**26**
NE	0	0	2	2
Not alive	0	1	2	2
Off trial	0	0	3	3
No data	0	4	2	2
Total	38	38	38	38

2 b) Evaluation of tumor markers at various clinical phases

A fall in biochemical tumor marker values towards normal is observed for the 38 patients during treatment, especially from the time of diagnosis to the end of induction therapy.

Figure 5: Tumor markers at various clinical phases

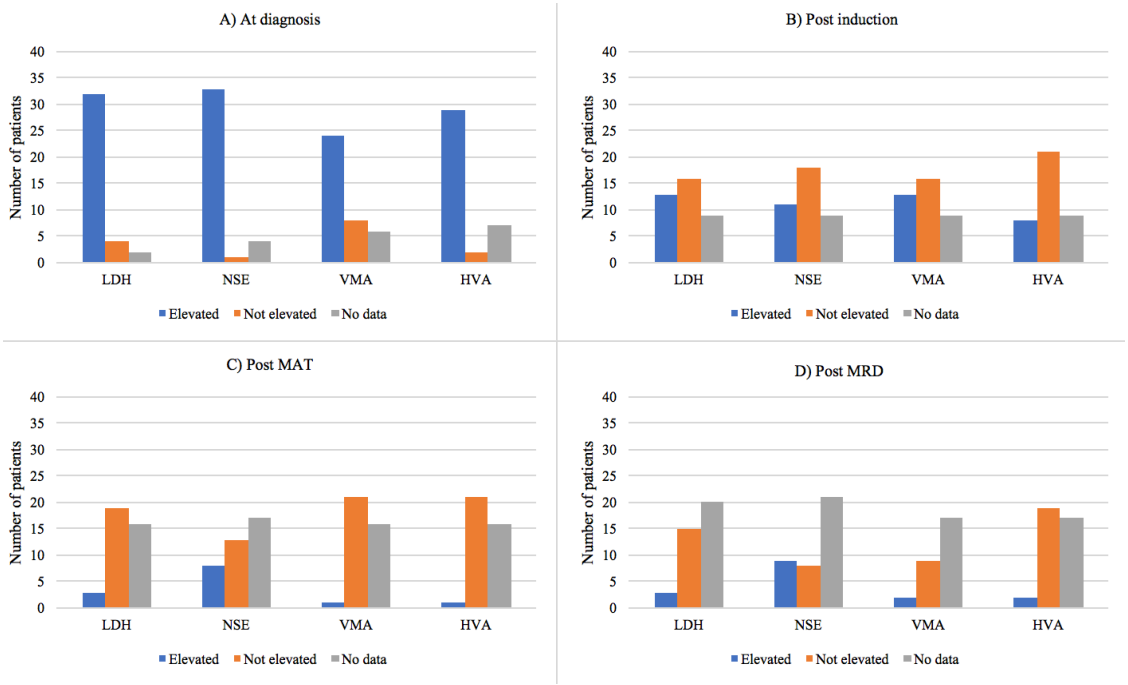


Table 8: Laboratory results at various clinical phase

Tumor marker	Reference value*	At diagnosis: Median (Range)	Post induction: Median (Range)	Post MAT: Median (Range)	Post MRD: Median (Range)
LDH (IU/I)	≤ 430	642 (139 – 5212)	264 (132 – 845)	209 (143 – 418)	249 (157 – 11000)
NSE (microg)	≤ 12	220 (12 – 1469)	11 (5 – 43)	11 (5 – 15)	12 (6 – 50)
VMA (µmol/mmol creat)	≤ 20	67 (2 – 460)	11 (2 – 39)	4 (2 – 29)	3 (2 – 10)
HVA (µmol/mmol creat)	≤ 20	67 (2 – 440)	9 (3 – 15)	6 (3 – 145)	6 (2 – 30)

**The normal values for LDH, VMA and HVA vary according to the age of the child. The normal range of values for NSE has changed over time, and for this present study it was set at ≤ 12 micrograms.*
The patients that had no data registered in the study database were excluded from this table.

A note should be made on the value for NSE that is elevated for a relatively high number of patients by the end of myeloablative treatment, when considering that most of the patients are in complete remission (CR) by this clinical phase. NSE can result elevated as a false positive with the presence of hemolysis, and this might be the cause of this observation made for the patients in remission in this study (also because the highest value for NSE after MAT is only 15 micrograms).

6.3 Part 3: Survival

It was desirable to investigate survival for the 38 children in relation to the prognostic factors of age at diagnosis and MYCN amplification. To give an overview, overall survival for the whole group of patients is here first analyzed.

3 a) Overall Survival

Figure 6 is a Kaplan-Meier survival curve showing the cumulative survival in years for all patients from the time of diagnosis to last follow-up date (set at 31.12.2017 for those patients that were still alive) or to the date of death.

The curve is two buckled with a steeper slope at the beginning that then flattens more out towards the end. This indicates that death occurred mostly with a shorter interval of time from the time of diagnosis, with the occurrence of death being less common as time advances from diagnosis. In total, 12 out of the 38 patients died.

The overall survival (OS) for the whole group of children is 81%, 74%, and 54% after three, five and ten years respectively.

Figure 6:

3 b) Age at diagnosis and survival

The Kaplan-Meier survival curve below intends to investigate whether there is a distinction in the probability of survival among the 38 patients in relation to their age at diagnosis.

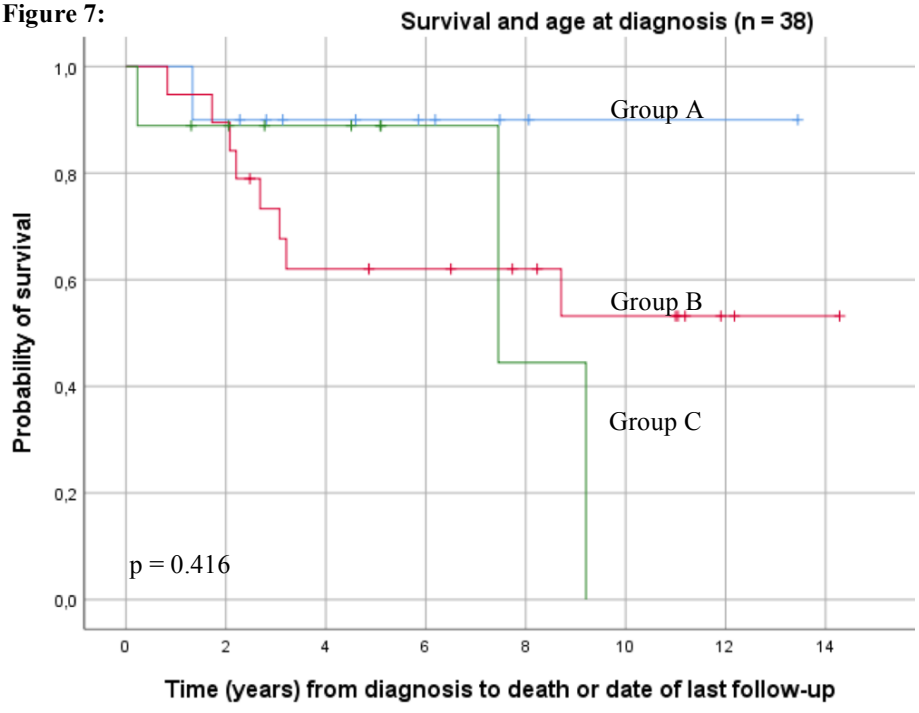
Figure 7 shows the cumulative survival from the time of diagnosis to last follow-up (31.12.2017) or date of death, with the children divided into groups by three age cut-off points: under or equal to 18 months (Group A = ten patients), over 18 months and under or equal to five years (Group B = 19 patients), and over five years of age (Group C = nine patients).

In Group A, one out of ten patients died. In Group B, eight out of 19 patients died. In Group C, three out of nine patients died.

In Group A (under 18 months) OS is 90%, 90% and 90% after three, five and ten years respectively.
In Group B (over 18 months and under or equal to five years) OS is 73%, 62% and 53% after three, five and ten years respectively.
In Group C (over five years) OS is 89%, 89% and 0% after three, five and ten years respectively.

Visually it appears that the children getting diagnosed with high-risk neuroblastoma at under 18 months of age have a better survival probability than older children There is however no statistical significance (p = 0.416) for the distinction in survival among the patients by the age cut-off points being tested in the figure below.

Figure 7:



3 c) MYCN amplification and survival

The Kaplan-Meier curve below intends to investigate whether there is a distinction in the probability of survival in relation to the presence or absence of MYCN amplification in the tumors of the children studied.

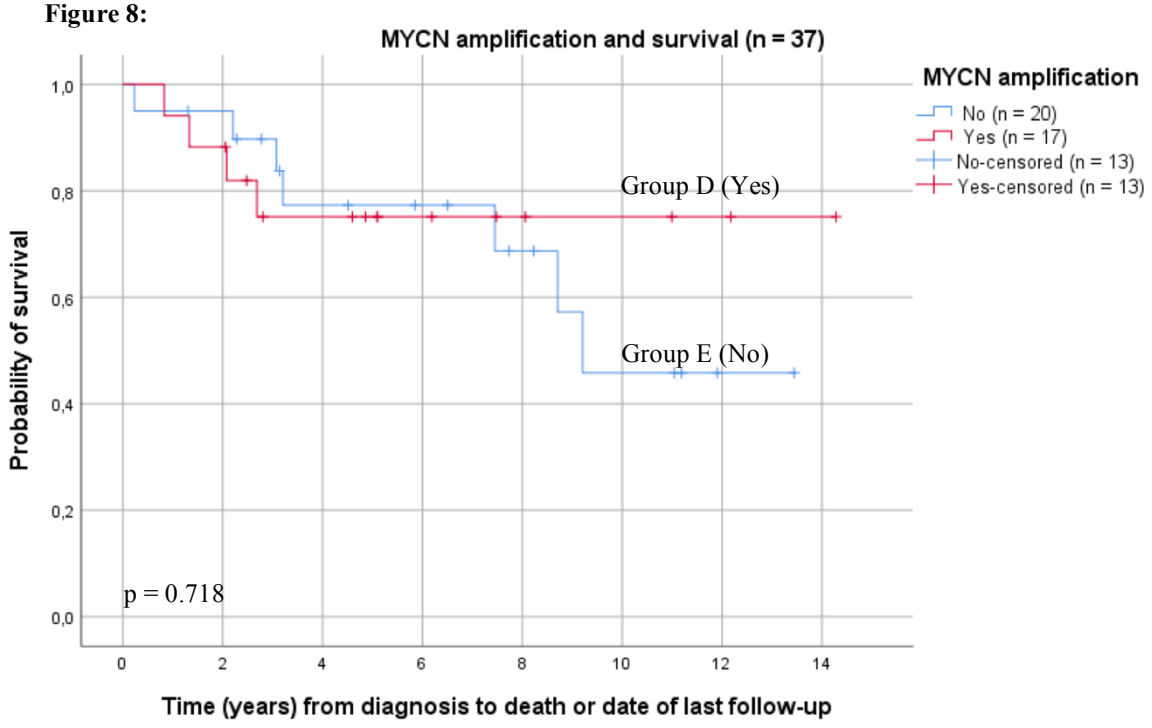
In figure 8, Group D (red) represents the children with the presence of MYCN amplification, while Group E (blue) represents the children with no MYCN amplification (any age).

In Group D, four out of 17 patients died. In Group E, seven out of 20 patients died.

In Group D (MYCN amplification) OS is 75%, 75% and 75% after three, five and ten years respectively.
In Group E (no MYCN amplification) OS is 90%, 77% and 46% after three, five and ten years respectively.

In figure 8, the two curves follow each other with a similar trend up to around two years form the time of diagnosis. At later points in time from diagnosis, only those children without MYCN amplification have occurrences of death. It appears that those with MYCN amplification that survive for around two years from diagnosis have a good or better prognosis. There is however no statistical significance (p = 0.718) in what is being tested in the figure below.

Figure 8:



1 of the 38 patients was excluded from this figure due to lack of data in the database.

7 Discussion

With this study, it was intended to give an overview of what characterizes the Norwegian children diagnosed with high-risk neuroblastoma and treated under the SIOPEN HR-NBL-1 study protocol (April 2003 – December 2015). The study included 38 children, predominantly boys, with high-risk neuroblastoma and median age of three years and three months. Most children had INSS tumor stage 4. Nearly all had elevated biochemical tumor markers at diagnosis, which disappeared during treatment in accordance with remission status. The overall 5-year survival rate of 74% was not significantly associated with age or MYCN amplification.

Patient characteristics at diagnosis

The age distribution in this study shows the early childhood peak typical in neuroblastoma. The incidence of neuroblastoma is said to be slightly higher among males than females, with the ratio of males to females being 11:8 in this study [11].The adrenal gland is the most common primary tumor site in neuroblastoma, also confirmed within this study. Histopathological findings (INPC) in the tumors studied go in line with high-risk features. The most common metastatic sites for the patients were bone marrow and skeleton, reflecting the classical sites for disseminated neuroblastomas [6].

Most of the patients in this study had INSS stage 4 disease. Cohn et al confirm the independent prognostic value of the INSS stage in an INRG database analysis, reporting superior overall survival for patients with non-metastatic disease and INSS stage 4S compared for patients with INSS stage 4 disease. In the newer INRG Staging System, genetic parameters as MYCN status and 11 q status are incorporated for their unfavorable prognostic impact on outcome [18]. MYCN is said to be amplified in 20% of neuroblastomas, with a great prevalence in stage 4 disease [20]. Among the known cases for MYCN status in this study, almost an even distribution was observed for the tumors in terms of a presence or absence of the amplification. All biochemical tumor markers (except ferritin) were elevated for most patients in this study. VMA levels tend to be relatively higher than HVA levels in children less than one year of age and in children with more favorable outcome [7].

Course of disease

According to the SIOPEN HR NBL-1 protocol, the disappearance of all detectable metastatic disease is among the few clinical factors that most consistently seem to have prognostic

significance in children treated with modern high-dose protocols. A good remission status by the end of induction is the most important prognostic factor related to the course of the disease, and the greatest improvement in bone and bone marrow remission status observed in this study was from the time of diagnosis to the end of induction. A positive change in terms of remission status for the group of children studied was also noticeable after high-dose chemotherapy (MAT), but the prognostic value of this observation is not clear. Results were considered good on the overall by the end of the last phase of treatment, with a good total of patients in complete remission for skeleton metastasis and most of the patients resulting negative for bone marrow involvement.

It would be of clinical interest to additionally explore results for primary tumor sites, in particular by the end of MAT phase, with the overall response from treatment being in fact established from results for both primary and metastatic sites [2]. However, this is not reported in this study due to lack of data for most of the patients.

VMA and HVA levels are said to fall to normal when tumor is surgically resected, and thus tumor recurrence or the progression of disease may be followed by comparing VMA and HVA to normal values [7]. An overall improvement with a fall in biochemical tumor markers towards normal during treatment was observed, with HVA and VMA levels already falling to normal by the end of induction therapy for most of the children. With the clinical picture of most patients being in remission by the end of myeloablative treatment, results for NSE are to be critically evaluated when observed to remain elevated for a relatively high number of patient. NSE is a sensitive factor, with the limitation of the occurrence of false positive values resulting from hemolysis due to the presence of enolase levels in erythrocytes and platelets [24].

Survival

The results for overall survival gave a 3-year OS of 81%, a 5-year OS of 74% and a 10-year OS of 54% for the group of patients studied as a whole. However, only 13/38 patients have been observed for ten years. The survival rates show rather satisfying figures and resulted higher than expected as long-term overall survival seen within international data for high-risk patients remain less than 50%, despite intensification of treatment over the last few decades [3]. It is though interesting to note that a tendency of an increase in survival rates for the later years in nearly all forms of childhood cancer is reported by the National clinical registry for childhood cancer, with a significance only obtained for children with neuroblastoma and other peripheral nerve-cell tumors. The National childhood cancer registry also reports that little change in

survival is seen after five years for children with neuroblastoma, suggesting that most relapse of disease occurs at earlier points in time [1]. Death from any cause, as the only endpoint investigated in this study, does evidently not reflect the whole clinical picture for children with high-risk neuroblastoma. A prospective study following the patients over a longer period of time would be more appropriate to investigate endpoints.

Age is a well-established prognostic variable in neuroblastoma, with an impact that is said to be continuous in nature. Historically, a cutoff of 365 days had been used as a surrogate for tumor behavior. Several retrospective studies specifically have examined whether 18 months might represent a more clinically relevant cut-off and demonstrated that patients with INSS stage 3 disease aged 12-18 months had a superior outcome to those older than 18 months [16, 25, 26]. To our knowledge, no new cut-off age for worsening prognosis has been published although SIOPEN has suggested five years to be the next cut-off age for this (unpublished SIOPEN data). For this reason, three age-cut off points were used in this study to test for a distinction in survival probability among the group of patients studied. The results for this were statistically not significant. It was however observed that children under 18 months had a better survival than the older children, with those of over five years giving the poorest results.

MYCN amplification is a well-established genomic marker of poor prognosis in neuroblastoma, and it was in this study tested for a distinction in survival for the presence or absence of MYCN amplification. The results for this were statistically not significant, however it appeared that those patients with MYCN amplification surviving two years from diagnosis had a better prognosis, with the occurrence of death in later clinical phases only seen in those patients without MYCN amplification. This trend is seen in the whole SIOPEN study when all countries are included, making it interesting to note that the protocol seems to compensate for MYCN amplification as a negative prognostic factor.

A number of articles evidence the importance of cytogenetic aberrations in identifying high-risk aggressive disease in MYCN non-amplified tumors [3, 9, 10]. Schleiermacher et al claim that a genomic profile defined by the presence or absence of specific segmental chromosomal alterations (as 17 q gain, 1 p deletion and 11 q deletion) will be clinically useful in risk stratification, in particular for neuroblastomas without MYCN amplification [9].

The fact is that there is no one gene identified for the tumorigenesis of neuroblastoma and the incorporation of gene therapy in new treatment strategies is claimed to be the move in finding

alternative, more efficient and more individualized treatment options for subgroups of patients [10].

Strengths and limitations

Strengths: This study is population based (looks at the entire nation), with a high inclusion rate in the protocol (we have no figures for this but the inclusion could be said to be according to the expected incidence of high-risk neuroblastoma in Norway). Data resulting from a multi-center study with a uniform standardized protocol and prospective online reporting is of a great advantage. The long observation time for those patients included in the first half of the inclusion period is a strength (although at the same time being a weakness for those patients included in the last part of the inclusion period, having a short observation time).

Limitations: Time to follow-up was limited for some, and death from any cause was the only endpoint studied, with the cause of death, relapse of disease, acute and chronic complications, and quality of life for long-term survivors not being considered. We did not have the opportunity to double-check information available in the database in the patients' records if missing or for valuation.

No statistically significant results could be drawn from the Kaplan-Meier survival curves plotted, with curves crossing at various points. Small subgroups for comparisons made within the sample of patients did clearly not contribute in reducing the likelihood of type II errors (false-negatives) occurring.

Conclusion and future projects

Neuroblastoma is known for its heterogeneity and although decades of analysis has resulted in a number of well-established prognostic variables, the case remains a tough nut to crack for pediatric oncologists. General observations and results for survival were in this study better than expected, however there is no statistical significance. A highlight of this study is the importance of international collaboration for Norway within this field of pediatric oncology, and children with high-risk neuroblastoma should continue to be enrolled on the SIOPEN study. Further international collaboration, including databases, is essential as new treatment strategies, based on specific tumor targets, are to be designed for subgroups of patients. The focus for future studies should include cause of death, complications from treatment and quality of life on the long term.

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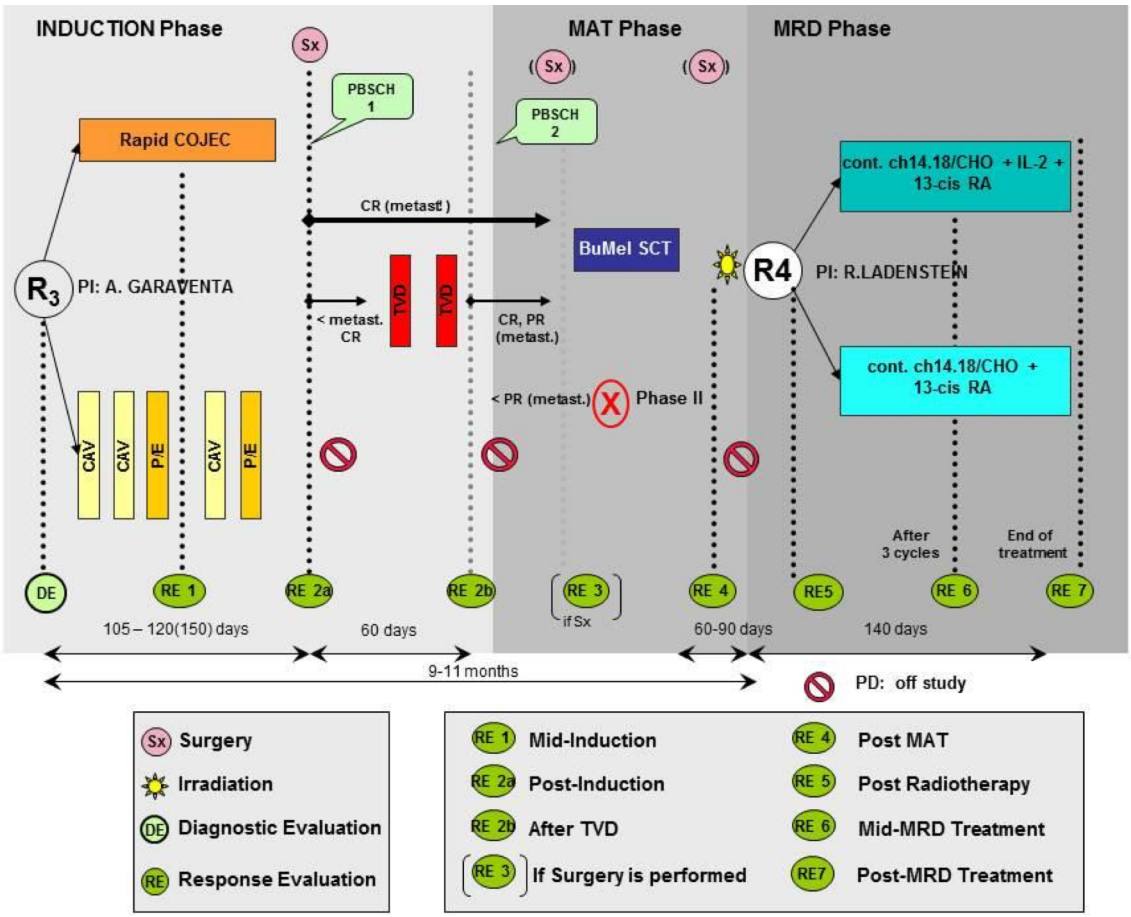
Appendix I

Trial Design

This is a randomised study of the European SIOP Neuroblastoma (SIOPEN) Group in high-risk neuroblastoma (stages 2, 3, 4 and 4s MYCN-amplified neuroblastoma, stage 4 MYCN non-amplified ≥ 12 months at diagnosis).

Figure 1: Treatment Summary Flow Chart HR-NBL1.5/SIOPEN



HRNBL1.7/SIOPEN valid per 04.04.2014 – R4 randomisation activated