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Case Report

Dilemmas in the Management of an Infant with Neuroblastoma Metastasized to the Muscles

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Neuroblastoma · Soft tissue metastasis · Risk stratification · Infant · Case report

Abstract
The risk stratification of infants with metastatic neuroblastoma (NB) has evolved over time from stage 4/M or IVs/4S/MS/Ms according to various staging systems. Despite these developments for some genetic aberrations, the prognostic value and the impact of soft tissue metastases in infants are not fully understood, nor well described in the different classification systems, hampering the definitions to uniformly treat patients and predict prognosis. A literature review on staging of infants with M/MS disease was performed at the occasion of the diagnosis of NB in an 8-month-old boy who presented with atypical metastatic sites in soft tissue and an aberrant tumor biology. The definitions of stage 4/4S/4s/M/MS/Ms were evaluated and compared to enable tumor risk stratification and inform management. International NB groups use different criteria for defining stage of infants with metastasized NB, resulting in differences in management. Limited literature is available on soft tissue metastases, especially muscular metastases, and is poorly incorporated into management guidelines mainly due to the lack of data. The uncertain prognosis of rare genetic aberrancies may add to the difficulties in treatment decisions. In some rare cases of NB in infants, the international treatment classification is not sufficient for staging and treatment decisions. Based on tumor progression, biology of unknown significance and a lack of evidence to classify a child under 12 months with NB and multiple muscular metastases, the patient was treated as stage 4/M and intermediate-risk protocols with a favorable outcome.

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Highlights

- Globally treatment approaches in infants with neuroblastoma and soft tissue metastases are not standardized.
- Metastases to the testis and muscles are rare and the prognosis not well documented.
- Metastases to the lymph nodes in infants have a favorable prognosis.
- The prognosis of soft tissue metastases in infants is influenced by biological characteristics.

Introduction

Neuroblastoma (NB) is a neuroendocrine malignancy of the adrenal glands and sympathetic system of mainly young children with a heterogeneous clinical presentation [1]. The management of each subclassification of NB is intricately linked to risk stratification systems that in turn are determined by patient and tumor characteristics [2, 3]. Each risk stratification is associated with the survival of patients diagnosed with NB [2, 3]. The main objective of the international neuroblastoma staging system is to enable comparison of different treatment strategies. Since this was a postsurgical staging system, it was later adapted to provide the clinician with a new pretreatment clinical risk group staging system (INRGSS) based on age, stage, presence/absence of image defined risk factors and on molecular tumor characteristics to guide treatment choice [3]. Despite these joined efforts, in rare cases, especially infants with metastatic disease, classification and treatment of NB may still differ according to the specific international treatment group because of different definitions and interpretations of the patient and tumor variables.

When presented with these difficulties during clinical staging and risk stratification in an infant with an unusual presentation of NB these incongruences will have implications on management and prognosis [4, 5]. We present an infant where limitations in international staging definitions in children less than 12 months diagnosed with NB posed difficulties in prescribing the appropriate treatment based on international NB protocols. Limited literature on soft tissue masses in infants with NB limited further management clarity.

Case Presentation

An 8-month-old male infant presented with bilateral large occipital-cervical lymph node adenopathy, hoarseness when crying and two hypo-echoic lesions demonstrated by ultrasound in segment II of an otherwise normal liver. A lymph node excision biopsy confirmed the diagnosis of NB. The infant was born at 36 weeks gestation without complications or other medical history of importance to NB pathology. On physical examination, the infant presented with the enlarged lymph nodes, was normotensive without organ enlargement, no skull lesions, no skin lesions or signs of bone marrow compromise such as anemia, infections, petechiae, or bleeding. No airway restriction or other life-threatening symptoms were present.

Laboratory investigations did not demonstrate cytopenia (Hb 10.7 g/dL, neutrophils 11.1 × 10^9/L, thrombocytes 244 × 10^9/L), with normal liver enzymes values, lactate dehydrogenase (401 U/L), and ferritin (35 μg/L). The 24-h urine catecholamine levels were increased: VMA/creatinine 112.5 mmol/mol (normal range 0.0–27.0 mmol/mol), HVA/creatinine 130 mmol/mol (normal range 0.0–33.0 mmol/mol), and dopamine 242 mmol/mol (normal range 0.0–85.0 mmol/mol).

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The histopathological examination of the cervical lymph node excision biopsy showed rosette structures of small blue round cells and differentiating neurogenic cells showing strong immunohistochemistry positivity for synaptophysin and ALK and focal positivity for CD57. Markers for rhabdomyosarcoma and Ewing sarcoma were negative. On a next-generation RNA sequencing, no gene rearrangement on the t(2p23) breakpoint of the ALK gene could be found.

An ^{123}I-mIBG-scan identified a right-sided cervical primary tumor with metastases to the contralateral cervical lymph nodes (shown in Fig. 1a) and mid-abdominal peritoneal lymph nodes (shown in Fig. 1b). On magnetic resonance imaging (MRI), the primary tumor measured 2.5 cm × 7.8 cm × 1.7 cm (volume of 17.2 cm^3) (shown in Fig. 1a). The ^{123}I-mIBG-scan further identified soft tissue metastases in the right intercostal muscles (shown in Fig. 1c, d) and vastus lateralis proximal muscle of the left upper leg (shown in Fig. 1e, f). The metastases were confirmed via computed tomography and/or MRI. The MRI also confirmed the liver metastases identified on ultrasound and an additional soft tissue metastasis of the back muscles on the left side of the thorax. Locoregional secondary mass-effect without encasement was demonstrated on the vascular venous structures of the right cervical region.

No bone lesions could be identified on X-rays, CT scan or 99Tc^m-bone scan. Bilateral bone marrow aspirates and trephine biopsies did not demonstrate the presence of malignant cells. Cytogenetic analysis revealed *MYCN* non-amplification and shallow whole-genome sequencing a del(14) (q23.3q32.12) segmental chromosomal aberration.

Risk Stratification

Various prognostic factors described in the literature did not inform a consensual stratification when considering the soft tissue metastasis (shown in Table 1) [6–9].

Staging, Treatment, and Patient Outcome

According to the International Society of Paediatric Oncology European Neuroblastoma group (SIOPEN) LINES protocol, the patient should be staged as MS: primary cervical tumor with metastatic disease present in liver and in soft tissue (lymph nodes and muscles). Stage M disease is defined as bone, pleural, lung, CNS metastasis, whereas MS disease is defined as metastatic disease confined to skin and/or liver and/or bone marrow (or even other sites such as lymph nodes and/or testes), in infants ≤12 months. Uptake of mIBG or technetium in the skeleton is not an exclusion criterion for MS, as long as the bone lesions are not visible by X-ray and/or CT scan. Metastatic disease in muscles is considered as soft tissue disease and is no exclusion criterion for Ms.

The allocation to a SIOPEN treatment risk group is determined by additional factors. No life-threatening symptoms or image defined risk factors were present. The tumor genomic profile revealed absence of MYCN-amplification, presence of numerical aberrations and of a segmental alteration (deletion 14 q), but the latter is considered as "no genomic profile result available" (based on the definition that if a segmental alteration is present but not of those observed recurrently in NB the profile is classified as no result). The recommendation is to give the standard treatment as for study group 4: observation only (shown in Table 2), per low-risk disease. However, in case the genomic profile was considered as SCA, the patient should be treated in LINES treatment group 6 with at least 4 × VP/Carbo, and if the metastatic muscle involvement would classify the infant as a M patient, as in other clinical trial groups (shown in Table 2), the patient should be treated according to LINES study group 10 with at least 4 × VP/Carbo (shown in Table 2).

Despite proposed allocation to LINES treatment group 4 (no treatment), chemotherapy was started according to SIOPEN-LINES intermediate-risk group, group 10, which is the same initial treatment as for treatment group 6. This decision was based on the uncertain significance of a structural chromosome abnormality, the somewhat older age of the patient (the prognosis for

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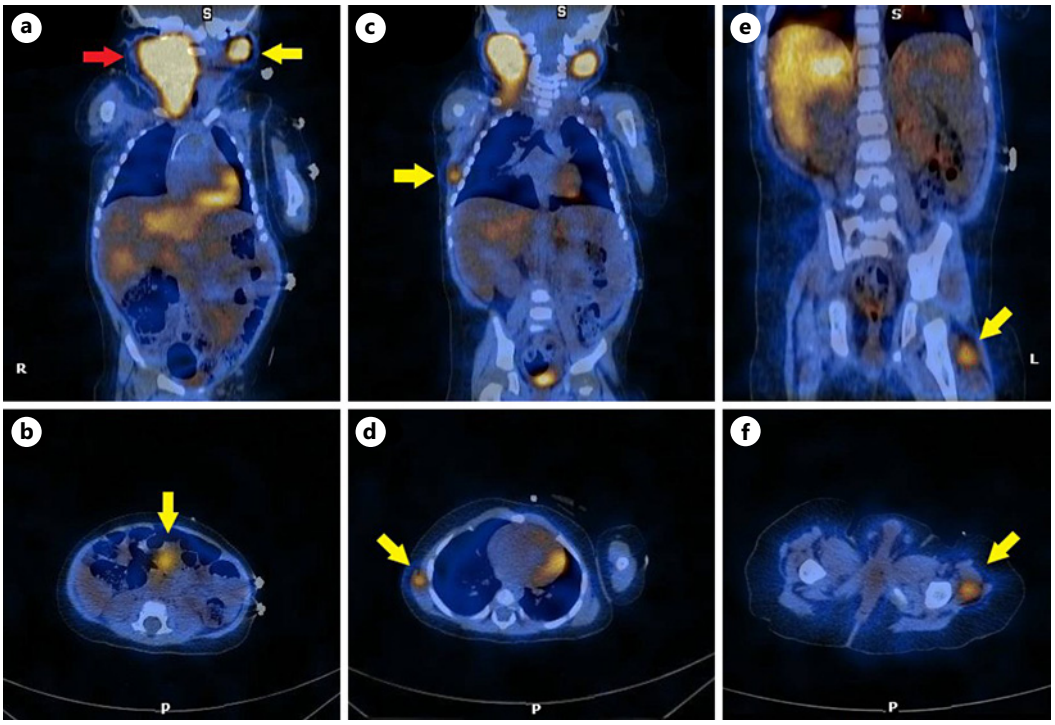

Fig. 1. The diagnostic *meta*-iodobenzylguanidine scans of the patient. **a** Sagittal view of the primary cervical neuroblastoma (red arrow) and contralateral metastasis to the lymph nodes (yellow arrow). **b** Transverse view of the abdominal metastasis (yellow arrow). **c** Sagittal view of the thoracic soft tissue metastasis (yellow arrow). **d** Transverse view of thoracic soft tissue metastasis (yellow arrow). **e** Sagittal view of the soft tissue metastasis (yellow arrow) in the upper left leg. **f** Transverse view of the soft tissue metastasis (yellow arrow) in the upper left leg.

infants >6 months) was described to be worse than for infants [10], the difficulty to decide on stage 4 or 4s and on top of these criteria, the clinical evolution with rapid tumor growth shortly after diagnosis. Treatment consisted of 4 × VP/Carbo and 4 × cyclophosphamide, adriamycin, vincristine because of persistent metastatic disease at first assessments. Thereafter, a favorable response from both the primary and metastatic sites was observed. The urine catecholamine levels normalized. Surgery was not attempted to remove the tumors in the neck, because it would be a mutilating procedure. At the end of therapy, the mIBG/CT-scan was in keeping with a differentiated primary tumor and contralateral neck lymph node that were reduced in size. All metastatic tumors were neither detectable by mIBG- nor CT-scan. The patient is still in remission more than 24 months after completing treatment. The CARE Checklist has been completed by the authors for this case report, attached as online supplementary material 1 (for all online suppl. material, see https://doi.org/10.1159/000531433).

Discussion

The distinction between stage 4/M and stage 4s/Ms in infants has treatment intensity implications and prognosis [7]. However, despite the international common efforts for determination of risk stratification an infant with metastatic disease might be treated as 4/M or as 4S/MS disease depending on the clinical trial the treating center is participating in (shown in online suppl. material 2).

Table 1. Neuroblastoma risk factors at diagnosis and risk prognostication

Risk factor at diagnosis	Patient factor	Quality of risk
Age at diagnosis	8 months	Good risk (with ≤6 months better than >6–12 months)
Primary tumor site	Neck	Good risk
Stage	4S or MS	Good risk
	4 or M	Poor risk
LDH	401 U/L	Good risk [7]
Ferritin	35 μg/L	Good risk [7, 8]
U-catecholamine levels	VMA/creatinine 112.5 mmol/mol	
	HVA/creatinine 130 mmol/mol	
	DA 242 mmol/mol	
	DA/creatinine 3,108 mmol/mol	
	VMA/HVA 0.87	Good risk [8]
	DA/VMA ratio 0.04	Poor risk [9]
Histology	Differentiating neurogenic cells	Good risk
	Favorable histology	
MYCN-amplification	Negative	Good risk
Segmental chromosomal aberrations	del(14) (q23.3q32.12)	Unknown
	No 1p or 11q aberrations were detected	Good risk [10]

DA, dopamine; HVA, homovanillic acid; LDH, lactate dehydrogenase; VMA, vanillylmandelic acid.

In 2009, the International Neuroblastoma Risk Group (INRG) defined a staging system (INRGSS) which could be used at diagnosis, before any treatment [1]. Since this publication attempts have been made to adapt the staging system and to refine treatment, stratification strategies taking into account more recent knowledge on robust genomic and molecular biomarkers. Despite international efforts to harmonize staging and determination of risk classification, the management of an infant with metastatic disease may differ from observation only to moderate or intense chemotherapy.

The Definition of Metastases in Stage 4s/Ms and M/4

According to the INRGSS stage 4s, 4S and MS the metastatic sites are defined as being limited to the liver, skin, and bone marrow. Bone metastasis is considered as stage 4/M and is defined by mIBG uptake in the skeleton. SIOPEN adapted the definition of bone metastasis as skeletal disease confirmed by X-ray or CT [11]. Infants under 12 months with disseminated disease without *MYCN*-amplification have excellent prognosis, even if mIBG uptake in the skeleton is present [12, 13]. This may reflect bone marrow disease but should be confirmed by a bone marrow aspirate. North American and Japanese protocols only require identification of bone metastases on nuclear studies without X-ray or CT confirmation.

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Table 2. Risk stratification and treatment based on SIOPEN (Europe), COG (North America), and DCOG (The Netherlands) protocols

Treatment group	Definition	Treatment
SIOPEN (Europe)		
Group 4	Ms patients with a NCA genomic profile, without life-threatening symptoms T	Observation
Group 6	Ms patients with a SCA genomic profile, with or without life-threatening symptoms	4 × VP16/carboplatin ± 2 x cyclophosphamide, adriamycin, vincristine (CADO)
COG (North America) version 2 risk stratification schema		
Low risk	Ms patients with a NCA genomic profile, without life-threatening symptoms T	MS score-based therapy (as in COG ANBL1232) – carboplatin etoposide
Intermediate risk	Ms patients with a SCA genomic profile, with or without life-threatening symptoms	Intermediate-risk therapy is response-based and involves two to eight cycles of chemotherapy, with maximal safe resection of residual primary tumors
Dutch childhood oncology Group (DCOG)		
Observation group	Ms patients, MYCN negative	Observation
Medium risk	M patient, <1 yr, MYCN negative	3 × N5 cycle (cisplatinum, etoposide, and vindesine) 3 × N6 cycle (vincristine, dacarbazine, ifosfamide, and doxorubicine), 4 × N7 cycle (low dose cyclophosphamide orally), 9 × retinoic acid 14-day cycles supportive care

SIOPEN-R-NET. European Low and Intermediate Risk Neuroblastoma Protocol: A SIOPEN Study Version 6.0; January 31, 2018: 53.

Within the INRGSS the presence of soft tissue metastasis in infants is not discussed separately, so implying stage 4 or M disease if present. In recent SIOPEN studies, however, the concept of including soft tissue metastatic sites in addition to the skin, liver, and bone marrow disease was added for the definition of 4S/MS disease. The soft tissue metastases may be divided into either lymph node or non-lymph node sites. The main non-lymph node sites include the gonads and muscles [14]. Subject of debate remains the contralateral lymph nodes, lymph nodes that cross the midline, and distant lymph nodes [1]. In perinatal cases of NB (less than 90 days), ipsilateral or contralateral regional lymph nodes and metastatic lymph nodes are exclusion criteria for observation [15]. Hero et al. analyzed 317 infants with metastatic NB and could not demonstrate a disadvantage in outcome for patients with distant lymph nodes [16]. Presence of contralateral lymph nodes was a poor significant prognostic factor only in patients managed with limited treatment, therefore they concluded that it was not advised to upstage from stage 4s to stage 4 on this basis alone [16]. The limitation of this study was that there was no uniform treatment applied in patient groups and ranged from no treatment to high-risk treatment [16]. Morgenstern et al. [17] reviewed the data of 2,250 international neuroblastoma staging system stage 4 patients, of whom 6.5% of the patients

had 4N disease. They reported that 85 (3.3%) stage 4N patients with metastatic disease limited to the lymph nodes, were of younger age (<18 months), and had mostly favorable biology. This subgroup had better outcome than older patients (≥18 months) with stage 4N metastatic disease ($p < 0.001$) and patients <18 months with non-4N metastatic disease ($p = 0.003$). The treatment also varied from no treatment or surgery only to advanced treatment with autologous stem cell transplant, yet 4N patients were less likely to receive intensive therapy than non-4N patients [17]. In our case report the contralateral lymph node was in the neck and not part of the same lymphatic drainage region. According to the current SIOPEN-LINES trial, our patient should be staged as Stage 4s/Ms and receive no treatment (treatment group 6) whereas according to the COG and GPOH trials he should be treated as M/4 patient.

There is a paucity of literature except single case reports on both primary and metastatic NB to the muscles [18–22]. It is hypothesized that in cases where the muscle is the only location that the patient has 4s disease with regression of the primary tumor and of the other metastatic disease or 4S NB. All the reported cases were less than 18 months of age and could be completely resected or responded favorably to chemotherapy [19–22]. Metastatic disease in muscle combined with other metastases is mostly associated with unfavorable molecular characteristics such as MYCN-amplification, 1p deletion, and gain of chromosome 17q [23, 24].

Criteria of Age for the Definition of 4 and 4s in Infants

For infants with metastatic disease, a different age cut off is used throughout the clinical trial groups [6]. In the SIOPEN group, without MYCN-amplification and in metastatic disease in the bone, pleura, lung, and/or CNS the patient needs to be treated as stage M disease, patients ≤12 months at diagnosis should be treated according intermediate-risk protocol recommendations, whereas patients >12 months are treated according to the SIOPEN-HR protocol. For Ms patients, a cut off of 12 months is used. For patients with localized L2 disease, an age cut off under 18 months is considered as low-risk disease, whereas L2 patients over 18 months are treated according to intermediate-risk treatment groups. According to the INRG 18 months is used as age cut off for definition of 4s/Ms disease in children younger than 18 months with metastases confined to the skin, liver, and/or less than 10% bone marrow involvement. Bone disease and any SCA, even those with unknown significance, are defined as HR independent of age.

The Impact of the Genomic Profile in Stage Ms/4s and M/4

The INRG project reported that the ratio of children under 18 months of age with SCA compared to those without was smaller than in children 18 months and older [6, 10]. Approximately, 34% of children less than 18 months with localized tumors have typical 1p/3p/4p/11q SCA and less than 3% had ploidy in combination with SCA [25]. Both of these lead to increased relapse rates. Metastatic disease is mostly associated with unfavorable molecular characteristics such as *MYCN*-amplification, 1p deletion and gain of chromosome 17q [23, 24]. Although Schramm et al. [23] reported that the overall tumor expression profiles between stages 4 and 4s were comparable.

The Differences between Treatment Protocols

The definition of Stage Ms does not distinguish between the lateralization and pattern of lymph node metastases. Yet the presence of *MYCN*-amplification in the presence of a stage Ms staging determines an upstaging in risk stratification [14]. This definition is supported by findings reported in the study by Morgenstern et al. [17]. In our case, only the age at diagnosis was favorable. The genetics had an uncertain prognostication and the implication of the two muscle metastases are not clearly defined in the literature neither in study group treatment protocols. Therefore, the stage 4N status remains the only factor with stratification value but does not confer a higher risk stratification on its own. Therefore, the guidelines for upstaging

our patient to stage 4 or M are not well defined. In the Dutch Childhood Oncology Group (DCOG), in collaboration with the German Gesellschaft for Pädiatrische Onkologie und Hämatologie (GPOH), the patient would only be observed if classified with stage 4s disease and would receive medium-risk treatment if classified with stage 4 disease. The Japanese are associated with SIOPEN and would receive, respectively, low-risk and intermediate-risk management is defined with stage 4s and stage 4 disease, respectively.

In protocols, where distant metastatic sites (excluding the liver, skin, and less than 10% bone marrow involvement) are clearly defined as stage 4 our case would receive higher intensity treatment compared to a stage 4S or 4s. In protocols utilizing stage MS and Ms, soft tissue metastases only fall into the two staging classification system where the literature and treatment protocols clearly define the role of the individual anatomic sites. In our case, the 12–18 month age exclusion was not relevant.

A solution for this problem would be to biopsy the metastases for inspection of cytological involvement and molecular profiling but different molecular profiles of primary tumors in 4S at the time of diagnosis have been described previously in NB, but not for metastatic sites [26]. However, in recurrent and relapse settings mutations have been described [27]. DNA methylation may be the only possibility to distinguish between stages, but this is not routinely performed. In this case, the muscle metastases were not biopsied because the mIBG-avid lesions are similar to primary NB and to avoid general anesthesia for a new biopsy.

Considerations Relating to Soft Tissue Metastases

The boy in our case did not have testicular involvement, but gonads are not systematically examined for tumor involvement, yet it is considered a sanctuary site [28]. Primary gonadal NB is only described in the literature in a dozen cases [29, 30]. Metastatic disease in gonads is rare, has generally poor outcomes, and has been associated with retroperitoneal abdominal primaries either through spread via blood or seeded from involved retroperitoneal lymph nodes [29–32]. Based on these rare reports, patients with metastatic gonadal soft tissue involvement under 18 months of age should not be treated as low-risk patients.

Although not present in this case report, only recommendations exist on the management of pleural and ocular metastases in infants [16]. Pleural metastatic disease is considered as stage 4/M disease in the SIOPEN group and thus a high-risk factor. Although other study groups recommend the same, it is not clearly incorporated their stratification systems.

Conclusion

The different stage and risk classification systems of NB stage 4/M and 4s/Ms disease in children under the age of 12 or 18 months poses challenges especially in case of an atypical primary and metastatic presentation at diagnosis. In this case report of an infant with NB, the metastatic pattern deviated from the standard definitions but had a good outcome with intermediate-risk treatment. Expert guidelines to define the application of soft tissue, especially muscular, metastasis should be formulated as insufficient data exists for evidence-based approaches.

Statement of Ethics

The Antwerp University Hospital Ethics Committee granted an exemption from requiring ethics approval for this case report. Written informed consent was obtained from the parents of the patient for publication of the details of the medical case and any accompanying images.

Conflict of Interest Statement

There is no conflict of interest.

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Author Contributions

J.H., G.L., and K.N. conceptualized the manuscript. J.H., G.L., K.N., M.A., N.R., and J.V. contributed and critically evaluated equally to the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding author.

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