The use of both diagnostic and therapeutic MIBG in neuroblastoma patients
Bleeker, G.

Citation for published version (APA):
Bleeker, G. (2014). The use of both diagnostic and therapeutic MIBG in neuroblastoma patients
Chapter 1

Introduction and outline of the thesis

Partly adapted from:
INTRODUCTION

Neuroblastoma is the most common extra-cranial malignant solid tumour of childhood (1-3). It accounts for 7 to 10% of all childhood cancers and for approximately 15% of cancer deaths in children (2-8). About 25 patients per year are diagnosed in the Netherlands. Ninety percent of patients are younger than five years of age at diagnosis with a median age of 22 months (6-9). Neuroblastoma is an embryonic tumour derived from the sympathetic adrenal lineage of the neural crest. Consequently, neuroblastomas may arise anywhere in the sympathetic nervous system, but most frequently they arise in the abdomen (65%) with half of them in the adrenal glands. Other common sites are the neck, chest and pelvis (3, 4, 10).

PRESENTATION

Clinical presentation depends on extent and site of disease. Patients can present with localised neuroblastoma at diagnosis, varying from incidentally diagnosed adrenal tumours to large locally invasive neuroblastomas with a large abdominal mass, abdominal distension and pain (3, 4). Cervical tumours may present with Horner’s syndrome (4, 11). Para-spinal tumours occur in 5 to 15% of neuroblastoma patients. If extending into the neural foramina, these tumours may cause myelum compression and thus neurological symptoms, like motor weakness, pain and sensory loss. These patients need immediate treatment, for example with chemotherapy, radiotherapy or laminectomy with similar results of overall outcome and of neurological symptoms. However, one should take into account that laminectomy may have serious orthopaedic consequences (12-15). Some patients develop hypertension due to compression of the renal vasculature by the primary tumour or due to increased catecholamine levels released by the tumour (4, 6).

METASTATIC DISEASE

Symptoms

Distant metastases are present at diagnosis in 50% of the patients (4, 10, 16). Dissemination occurs through lymphatic and haematogenous routes and tumour cells affect predominantly the bone, bone marrow and lymph nodes and less frequently the liver and lungs (4, 10, 16). Children with metastatic disease are frequently quite ill at presentation. As the tumour disseminates to the skeleton, patients often present with bone pain, limping or both. Patients may show anaemia at diagnosis because of marrow failure or tumour bleedings. Other typical signs are peri-orbital ecchymosis (raccoon eyes), with or without proptosis, caused by metastases in the bony orbit. Orbital metastases are characteristic for neuroblastomas and are uncommon in other forms of childhood cancer.
In the group of children with orbital metastases, neuroblastomas account for the greater part (3, 4, 6, 17, 18).

**Biology**

The formation of metastases progresses by a multi-step process, often called the invasion-metastases cascade (19-21). The first step is invasion of primary tumour cells in the surrounding tissue. Secondly, cancer cells intravasate into and travel by the bloodstream and lymphatic systems. The next step is extravasation of cancer cells from these vessels into the parenchyma of distant tissues. Finally, colonisation of distant tissues results in the formation of metastases (19-21). Epithelial-mesenchymal transition (EMT) also plays a role in the metastatic process. EMT is a highly conserved cellular program that allows polarised, non-motile epithelial cells to acquire a mesenchymal state with more invasive, metastatic capacity (22, 23). Already in 1889, Stephen Paget suggested that metastases are not consequent on chance, but grow (“seed”) preferentially in the microenvironment of selected organs (the “soil”). Metastases will develop if the appropriate “seed” is implanted in “suitable soil” (24, 25). With regard to bone metastases, the bone is a particularly fertile “soil”, because it contains a lot of immobilised growth factors such as transforming growth factor β (TGF β), insulin-like growth factors I and II (IGF I and II), fibroblast growth factor (FGF) platelet-derived growth factor (PDGF) and bone morphogenic proteins (BMP). Upon bone degradation these growth factors are released, they become activated and they stimulate tumour cells and osteoblast proliferation, and release of parathyroid hormone related peptide (PTHrP) (26).

Bone metastases can be classified into the osteoclastic and osteoblastic type, although in most cases both types play a role. Neuroblastomas develop osteoclastic (osteolytic) metastases. There are multiple pathways by which neuroblastoma cells can activate osteoclasts, for instance by the expression of RANKL or through secretion of IL-6 by bone marrow mesenchymal stem cells (26).

**STAGING**

Staging of neuroblastomas was previously performed according to the International Neuroblastoma Staging System (INSS) (Table 1) (27, 28). Stages 1 to 2 neuroblastomas were localised, stage 3 tumours showed only regional disease and stage 4 neuroblastomas included the presence of distant metastases. A unique pattern of metastases, limited to the liver, skin and less than 10% of bone marrow in children younger than one year, is defined as stage 4S, which has a potential for spontaneous regression. Because the INSS system is a postsurgical staging system, in 2008 the International Neuroblastoma Research Group (INRG) published a new clinical staging system that uses image defined risk factors on computed tomography (CT) or magnetic resonance
Chapter 1

Introduction and outline of the thesis

Table 1: Description of original International Neuroblastoma Staging System

<table>
<thead>
<tr>
<th>Stage</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Localised tumour with complete gross excision with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumour microscopically (nodes attached to and removed with the primary tumour may be positive).</td>
</tr>
<tr>
<td>2A</td>
<td>Localised tumour with incomplete gross resection; representative ipsilateral non-adherent lymph nodes negative for tumour microscopically.</td>
</tr>
<tr>
<td>2B</td>
<td>Localised tumour with or without complete gross excision with ipsilateral non-adherent lymph nodes positive for tumour; enlarged contralateral lymph nodes must be negative microscopically.</td>
</tr>
<tr>
<td>3</td>
<td>Unresectable unilateral tumour infiltrating across the midline(^a) with or without regional lymph node involvement, localised unilateral tumour with contralateral regional lymph node involvement, or midline tumour with bilateral extension by infiltration (unresectable) or by lymph node involvement.</td>
</tr>
<tr>
<td>4</td>
<td>Any primary tumour with dissemination to distant lymph nodes, bone, bone marrow, liver, skin or other organs (except as defined for stage 4S).</td>
</tr>
<tr>
<td>4S</td>
<td>Localised primary tumour (as defined for stage 1, 2A or 2B) with dissemination limited to skin, liver or &lt;10% of bone marrow (limited to infants &lt; 1 year of age).(^b)</td>
</tr>
</tbody>
</table>

Multifocal primary tumours (e.g. bilateral adrenal primary tumours) should be staged according to the greatest extent of disease, as defined in the table, and followed by a subscript ‘M’ (e.g. 3M).

\(^a\) The midline is defined as the vertebral column. Tumours originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.

\(^b\) Marrow involvement in stage 4S should be minimal (i.e. < 10% of total nucleated cells identified as malignant on bone marrow biopsy or marrow aspirate). More extensive marrow involvement would be considered to be stage 4. The MIBG scan (if performed) should be negative in the marrow.

Source - reference (27, 28).

Table 2: Description of International Neuroblastoma Risk Group Staging System (57)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>Localised tumour not involving vital structures as defined by the list of image-defined risk factors and confined to one body compartment.</td>
</tr>
<tr>
<td>L2</td>
<td>Loco regional tumour with presence of one or more image-defined risk factors.</td>
</tr>
<tr>
<td>M</td>
<td>Distant metastatic disease (except stage MS).</td>
</tr>
<tr>
<td>MS</td>
<td>Metastatic disease in children younger than 18 months with metastases confined to skin, liver and/or bone marrow.</td>
</tr>
</tbody>
</table>

Patients with multifocal primary tumours should be staged according to the greatest extent of disease as defined in the table.

Source – reference (57).

Imaging (MRI) instead of surgical evaluation (Table 2) (29, 30). Image defined risk factors include the involvement of vital structures with the risk of injury to these structures during surgery (30). In the INRG staging system, stage L tumours are localised; L1 tumours do not involve vital structures as defined by the image defined risk factors and the tumour must be confined to one body compartment; L2 tumours are loco-regional tumours with
one or more image defined risk factors and the tumour may extend in adjacent body compartments ipsilaterally. Stage M tumours show distant metastatic disease and in stage MS tumours, as in stage 4S, metastases are confined to the skin, liver and bone marrow (less than 10%) in patients younger than 18 months (29).

**PROGNOSTIC FEATURES**

Neuroblastoma is a tumour with very heterogeneous clinical and biological behaviour. The clinical course ranges from spontaneous regression to rapid and fatal tumour progression despite extensive treatment.

Current clinical and biological prognostic markers used for treatment stratification of neuroblastoma patients are INSS or INRG stage, age at diagnosis, histology, MYCN amplification, chromosome 1p aberration, chromosome 11q aberration, chromosome 17q gain and DNA content (1, 4, 27, 28, 31-34).

Children younger than 18 months have a better prognosis than older children. Histopathological assessment of neuroblastomas is commonly performed according to the International Neuroblastoma Pathology Classification system (INPC), which uses the Shimada classification (Table 3). Neuroblastoma patients with tumours classified as ganglioneuroma maturing type and ganglioneuroblastoma intermixed type have a better prognosis than patients with tumours classified as ganglioneuroblastoma nodular type and neuroblastoma type (35-37).

The most common genetic alteration in sporadic neuroblastoma is the amplification of MYCN (≥ ten copies for diploid genome or > fourfold signal relative to chromosome 2). Around 22% of neuroblastomas show MYCN amplification, which is associated with poor outcome (1, 38). MYCN regulates proliferation, growth, differentiation and survival of cells in the developing central nervous system. When overexpressed, MYC oncoproteins (transcription factors) can lead to deregulated growth and proliferation (1, 38). It strongly predicts outcome in stage 1 to 3 and 4S neuroblastomas, but its prognostic significance is less clear in stage 4 disease (4, 16, 39, 40).

Deletion of the short arm of chromosome 1 (1p) (loss of heterozygosity of 1p (1pLOH)) is a common abnormality in patients with advanced stages of disease (25 to 35% of neuroblastomas). It is highly associated with MYCN amplification and with an unfavourable outcome (1, 41).

Chromosome 11q loss is observed in 35 to 45% of neuroblastomas. It is not frequently observed in MYCN amplified tumours, but it correlates with other high-risk features (34, 42). Chromosome 17q gain is often caused by an unbalanced translocation with chromosome 1 or 11 and correlates with more aggressive tumour behaviour. The prognostic significance of 17q gain relative to other clinical-biological parameters is still under investigation (4).
The presence of bone marrow and bone metastases has been shown to be independent adverse prognostic factors in metastatic neuroblastoma, next to age and MYCN-amplification (MNA) (43-47). Many studies report different outcomes for patients with bone and/or bone marrow metastases (43, 45, 47). In patients with metastatic disease younger than one year at diagnosis, the presence of bone metastases leads to a worse outcome than the sole presence of bone marrow metastases (44, 46).

The current risk classification system of the INRG uses stage, age at diagnosis, histology, MYCN amplification, 11q aberration and DNA content to identify four risk groups: very low, low, intermediate and high, with event-free survivals (EFS) of >85%, 75 to 85%, 50 to 75% and <50%, respectively (33).

<table>
<thead>
<tr>
<th>Category:</th>
<th>Favourable:</th>
<th>Unfavourable:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade of differentiation</td>
<td>MKI</td>
</tr>
<tr>
<td>Neuroblastoma (Schwannian stroma-poor)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age&lt;18 months</td>
<td>Poorly differentiated</td>
<td>Low or intermediate</td>
</tr>
<tr>
<td></td>
<td>Differentiating</td>
<td>Low or intermediate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 18 – 60 months</td>
<td>Differentiating</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>Poorly differentiated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Differentiating</td>
<td>Intermediate/high</td>
</tr>
<tr>
<td>Age ≥ 60 months</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ganglioneuroblastoma intermixed (Schwannian stroma-rich)</td>
<td>All cases</td>
<td>-</td>
</tr>
<tr>
<td>Ganglioneuroma (Schwannian stroma-dominant)</td>
<td>All cases</td>
<td>-</td>
</tr>
<tr>
<td>Ganglioneuroblastoma nodular (composite, Schwannian stroma-rich, -poor, -dominant)</td>
<td>Favourable subset</td>
<td>Unfavourable subset</td>
</tr>
</tbody>
</table>

MKI: mitosis-karyorrhexis index, low < 100/5000 cells, intermediate 100-200/5000 cells, 200/5000 cells. Source – reference (30, 35-37)
BIOLOGY AND GENETICS

Familial neuroblastoma is rare (<2% of all neuroblastomas). Germline mutations in the anaplastic lymphoma kinase (ALK) gene on chromosome 2p23 were discovered to be present in about 50% of familial neuroblastomas. This makes ALK the major familial neuroblastoma predisposition gene, in addition to the known PHOX2B gene (10, 48). PHOX2B mutations explain only a small subset of hereditary neuroblastoma and almost exclusively in patients with associated disorders as congenital central hypoventilation syndrome and Hirschsprung’s disease (10, 38, 49-51). Approximately 6 to 10% of sporadic neuroblastomas also carry somatic ALK-activating mutations and an additional 3 to 4% have a high-level of ALK gene amplification. These are associated with poor outcome, especially in the presence of MYCN amplification (38).

Although some patients with neuroblastoma have a predisposition to neuroblastoma, the majority of these tumours occur spontaneously. Somatic changes, such as gain of alleles and activation of oncogenes, loss of alleles or changes in tumour-cell ploidy, play an important role in the development of sporadic neuroblastomas (1). Recently, a whole-genome sequence analysis of 87 neuroblastomas of all stages was performed. Only a few gene defects were identified. Two novel molecular defects were revealed: chromothripsis (local shredding of chromosomes) and neuritogenesis gene alterations, that frequently occur in high-risk neuroblastoma (52).

There is an important association between ATRX mutations and age at diagnosis of neuroblastoma. Patients younger than 18 months of age with stage 4 disease have a better prognosis than their older counterparts. No ATRX mutations have been identified in the younger patients, but in the older patients, especially those older than 12 years (with accordingly a very poor prognosis) ATRX mutations were found. Although the relationship between age at diagnosis and ATRX mutations was significant, the prognostic impact of ATRX mutations is not yet clear (38, 52). The tumour-driving genes CCND1 and CDK4 show copy number gains or high-level amplifications. High expression of CDK4 is associated with unfavourable prognosis (52-54).

DIAGNOSIS

Initial assessment of all patients suspected of neuroblastoma should include histopathological confirmation of the diagnosis and determination of tumour characteristics and extent of the disease, necessary for risk classification. This should be performed prior to any therapy and consists mostly of (histo-)pathology and imaging.

Pathology

The recommended criteria for diagnosing neuroblastoma according to the INSS are:
1. an unequivocal pathological diagnosis (INPC) from tumour tissue (with or without
immuno-histology, increased urinary catecholamines or metabolites); or 2. a bone marrow aspirate or trephine biopsy containing unequivocal tumour cells and increased urinary catecholamines or metabolites (28, 29). Bone marrow metastases are assessed by bone marrow aspirates from at least four different puncture sites (bilateral posterior iliac crest, two left and two right) with at least one single positive site, which is determined by morphology on smears and biopsies (28, 29).

**Imaging techniques**

To evaluate the primary tumour, international consensus was achieved in the INRG. MRI and/or CT with three-dimensional measurements are recommended (29). These two imaging modalities are necessary to address image-defined risk factors (IDRF). In addition, metastatic sites should also be measured by CT and/or MRI, because the results are useful for evaluation of treatment response (29). Furthermore, all patients should have $^{123}$I-MIBG scintigraphy according to the guidelines of the European Association of Nuclear Medicine (EANM) (55, 56). One unequivocal MIBG-positive lesion at a distant site is sufficient to define metastatic disease. A single dubious lesion on MIBG scintigraphy requires confirmation by another imaging modality (57).

**Ultrasound**

Ultrasound is usually the first imaging technique performed, because it is widely available and non-invasive. It allows accurate localisation of the primary tumour and the relationship with adjacent organs and vessels. However, it has important limitations: a low interobserver reproducibility and limited assessment of highly calcified tumours because of acoustic shading. More detailed imaging is required for adequate treatment planning by surgeons and radiation oncologists. Therefore MRI and CT are recommended at the time of diagnosis for accurate staging (30).

**Computed tomography (CT)**

CT is widely available and fast acquisitions reduce the need for sedation. However, it is associated with high radiation burden as children have a higher sensitivity to the negative effects of ionizing radiation.

**Magnetic resonance imaging (MRI)**

MRI has a higher contrast resolution and no radiation exposure, vessels can be visualised without the use of contrast material and it can display the spinal cord in high detail. Disadvantages are the limited availability of MRI in some countries and the need for sedation, because of its longer acquisition time (30).
MRI is reported to be able to detect bone as well as bone marrow metastases, but the role in patients with neuroblastoma is not yet clear. It has a unique soft tissue contrast resolution enabling early assessment of bone marrow infiltration by the tumour before osseous destruction becomes apparent on radiography or CT or metabolic changes occur on bone scintigraphy or Fluorine-18-fluorodeoxyglucose positron emission tomography (18F-FDG-PET) (61-63).

Generally, a combination of unenhanced T1-weighted spin echo and turbo-short TI inversion recovery (STIR) sequences is used for the detection of bone marrow metastases. On T1-weighted sequences normal fat containing marrow is replaced, resulting in a hypo-intense signal. In contrast to this, on STIR, a fat-suppressed sequence, lesions are visualised as hyper-intense signals due to increased content of water within the tumour cells (64). However, distinction between bone and bone marrow metastases is difficult, because, in contrast to adults, in children it is difficult to differentiate highly cellular hematopoietic marrow (red marrow) from metastatic disease. Therefore, it is important to have knowledge of age-dependent conversion patterns of red to yellow bone marrow. Other pitfalls are false-positive results caused by post-therapy marrow signal alterations due to oedema, necrosis, fibrosis or red marrow hyperplasia. (61;62;65;66)

Metaiodobenzylguanidine (MIBG) scintigraphy

MIBG is a compound that is structurally analogous to the neurotransmitter norepinephrine. It is actively taken up in neuroendocrine cells via the norepinephrine transporter (NET) and is stored in the neurosecretory granules, resulting in a high specific concentration in contrast to cells of other tissue (58-61). Once labelled with radioactive iodine (123I or 131I), MIBG scintigraphy can be used for imaging tumours of neuroendocrine origin like neuroblastomas (58-62). Initially MIBG was labelled with 131I, followed by 123I. 123I-MIBG scintigraphy results in superior image quality compared to 131I-MIBG at a lower patient radiation burden. The first explanation for this is that 123I-MIBG has a shorter half-life than 131I-MIBG (13 hours vs 8 days). Secondly, 123I-MIBG has a lower gamma-emission energy (159 keV) than 131I-MIBG (364 keV) making it more ideal for gamma camera imaging. Finally, 123I-MIBG lacks bêta particle emission (55, 56, 58, 60, 63, 64). Although image quality is better for 123I-MIBG, it has been shown that both radiopharmaceuticals perform equally the prediction of outcome using a semi-quantitative scoring system (64). However, the physical characteristics that make 131I-MIBG less suitable for imaging, make it a good candidate for radionuclide therapy of neuroblastoma.

The physiological distribution of MIBG consists of accumulation of the radiopharmaceutical in structures that excrete catecholamines, like the bladder, urinary tract and gastro-intestinal system. MIBG is also physiologically taken up by the liver and in a lesser extent by the spleen, lungs, salivary glands, thyroid, hypophysis, skeletal muscles myocardium
and brown adipose tissue. It is essential to be aware of this physiological distribution to avoid false-positive interpretation of MIBG scans (55, 60, 62). MIBG scintigraphy is both highly sensitive (88-93%) and highly specific (83-92%) (63). SPECT increases the accuracy of localisation of the primary tumour and metastases and it enables to differentiate lesions from areas of physiological uptake (65). About 10% of neuroblastomas are MIBG-non-avid causing false negative results. Pharmacological interference is probably the most frequent cause. Many drugs can interfere with the uptake and/or vesicular storage of $^{123}$I-MIBG, or both (55, 60, 62). For example, a substantial proportion of neuroblastoma patients presents with hypertension and has to be treated with antihypertensive agents. Several antihypertensive agents used in children, like labetolol, interfere with MIBG-uptake. It is recommendable to stop all interfering medication before the imaging procedure (55, 60). However, in case of severe hypertension this is not possible and usually the antihypertensive agent is changed into one that does not decrease uptake of MIBG (55, 60, 62). Furthermore neuroblastomas can be MIBG-non-avid because of low expression of the norepinephrine transporter (58, 66). Both MIBG-avid and MIBG-non-avid lesions can be present in the same patient and at the same time. The reasons for these discrepancies are unknown, but cell characteristics may play a role in MIBG-non-avidity.

To establish tumour load at diagnosis for prognostic reasons and treatment planning and to evaluate response during treatment, semi-quantitative scoring systems for the analysis of MIBG scans were developed (Table 4). The two most commonly used scoring systems, the scoring system of the International Society of Paediatric Oncology European Neuroblastoma Research Network (SIOPEN) and the Curie-system, divide the skeleton in anatomical segments, and give each segment an individual score for extension (quantity of metastases) and intensity (relative uptake compared to background or reference organ) (65). Both scoring systems have a good interobserver concordance. The Curie-system has been reported useful to correlate with overall-survival (OS) and event-free survival (EFS) (65, 67).

Although bone and bone marrow metastases are reported as distinct prognostic factors within metastatic neuroblastoma, these scoring systems do not distinguish between the two types of metastases. As the term of the Curie-scoring system already reveals, this is a (semi-) quantitative scoring system. Qualitative aspects, like the pattern of metastases, that might play a role in the metastasising process, are not taken into account. In contrast, the SIOPEN-system differentiates between focal discrete lesions and diffuse infiltration, but does not assign these patterns to bone or bone marrow metastases.

**Technetium-99m-methylene diphosphonate ($^{99m}$Tc-MDP) bone scintigraphy**

For patients with proven neuroblastoma without MIBG-uptake at diagnosis, $^{99m}$Tc-bone scintigraphy (bone scintigraphy) has long been the standard test to evaluate skeletal
Chapter 1

Metastases (63). However, bone scintigraphy is a highly sensitive modality for metastases of the osteoblastic type, and far less sensitive for osteolytic metastases. Bone metastases of neuroblastomas are generally of the osteoclastic type and therefore bone scintigraphy may underestimate skeletal involvement especially in the presence of smaller lesions (68). As a result, bone metastases of neuroblastoma are only depicted at a more advanced stage of the disease, when bone remodelling takes place (66). Furthermore, elevated uptake is present in the normal growth plates of children, making it difficult to assess the presence of bone metastases in these regions (60;67). Therefore alternative tests are investigated.

Table 4: Overview of MIBG scoring methods

<table>
<thead>
<tr>
<th></th>
<th>Curie semi-quantitative</th>
<th>SIOPEN semi-quantitative / qualitative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of skeletal body segments</strong></td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>- head and face</td>
<td>- skull</td>
</tr>
<tr>
<td></td>
<td>- neck and back vertebral column</td>
<td>- thoracic cage</td>
</tr>
<tr>
<td></td>
<td>- ribs and sternum</td>
<td>- proximal right upper limb</td>
</tr>
<tr>
<td></td>
<td>- lumbar and sacral column</td>
<td>- distal right upper limb</td>
</tr>
<tr>
<td></td>
<td>- pelvis</td>
<td>- proximal left upper limb</td>
</tr>
<tr>
<td></td>
<td>- arms</td>
<td>- distal left upper limb</td>
</tr>
<tr>
<td></td>
<td>- fore arms and hands</td>
<td>- spine</td>
</tr>
<tr>
<td></td>
<td>- thighs</td>
<td>- pelvis</td>
</tr>
<tr>
<td></td>
<td>- legs and feet</td>
<td>- proximal right</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- lower limb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- distal right lower limb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- proximal left lower limb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- distal left lower limb</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Soft tissue</strong></th>
<th>Yes</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quantitative score</strong></td>
<td>Extension score (0-30): 0: no sites per segment; 1: one site per segment; 2: more than one site per segment; 3: diffuse involvement (&gt;50% of the segment). Intensity score (0-30): 0: no uptake; 1: doubtful uptake; 2: definite uptake less than liver; 3: intense uptake greater than that of liver</td>
<td>Extension score (0-72): 0: no involvement; 1: one discrete lesion; 2: two discrete lesions; 3: three discrete lesions; 4: 3 discrete foci or a single diffuse lesion involving &lt;50% of a bone; 5: diffuse involvement of ≥50% to 95% of whole bone; 6: diffuse involvement of the entire bone.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Qualitative score</strong></th>
<th>No</th>
<th>Discrete foci v diffuse lesions.</th>
</tr>
</thead>
</table>

SIOPEN: International Society of Paediatric Oncology European Neuroblastoma Research Network;
Fluorine-8-fluorodeoxyglucose positron emission tomography (\textsuperscript{18}F-FDG-PET)-CT  
One promising imaging technique for (MIBG-negative) neuroblastoma is \textsuperscript{18}F-FDG-PET-CT. This integrated imaging method can reveal malignant bone marrow infiltration in an early stage based on its increased glucose metabolism. The combined, morphologic information with metabolic changes makes this technique highly sensitive and specific. Because the brain massively accumulates \textsuperscript{18}F-FDG, metastatic lesions in the skull cannot be viewed adequately. As in the assessment of MIBG imaging, one should be aware of physiologic distribution of the radiopharmaceutical, such as accumulation in brown adipose tissue or inflammation. Cytokine-mediated diffusely hyper-metabolic bone marrow may also give false-positive results as with the use of G-CSF and erythropoietin (69). Data on \textsuperscript{18}F-FDG-PET-CT in patients with neuroblastoma are still limited and it is mostly used in patients with neuroblastomas that do not accumulate or weakly accumulate MIBG (68). Most studies report that \textsuperscript{123}MIBG scintigraphy is superior to \textsuperscript{18}F-FDG-PET scintigraphy in the evaluation of stage 4 neuroblastoma due to its better detection of bone and bone marrow metastases (69-73). In stage 1 and 2 neuroblastoma \textsuperscript{18}F-FDG-PET scintigraphy detected more sites of primary disease and loco-regional metastases. In MIBG-non-avid stage 3 and 4 neuroblastoma it better detected disease extend in the chest, abdomen and pelvis (69, 73, 73). One study reported that in the absence or after resolution of cranial vault lesions, and once the primary tumour was resected, PET and bone marrow tests suffice for monitoring neuroblastoma patients at high risk for progressive disease in soft tissue and bone/bone marrow (74).

Indium-\textsuperscript{111}-Octreotide/pentetreotide (somatostatin) scintigraphy  
The sensitivity of \textsuperscript{111}In-octreotide scintigraphy ranges from 55-70%, much lower than \textsuperscript{123}I-MIBG imaging, and the specificity ranges from 83-94% (75). However, this imaging technique might be helpful in MIBG-negative neuroblastoma (76).

TREATMENT

Patient with neuroblastoma are treated according to risk groups (Figure 1). In patients with low-risk neuroblastoma a wait-and-see policy might fulfil, while high-risk patients are treated with dose-intensive regimens.

Low-risk and medium-risk neuroblastoma  
Most localised neuroblastomas (INSS stage 1-2, INRG stage L1) have favourable clinical biological features. Some do not need any treatment, because they spontaneously regress. Most other localised tumours can be treated with surgery alone (Figure 2) (4, 77).

Treatment of more invasive loco-regional tumours (INSS stage 3, INRG stage L2) is controversial (Figure 3). Chemotherapy is administered to enable surgical resection (13-15,
Since the prognosis of these loco-regional neuroblastomas is good, toxicity and late effects should be prevented as much as possible, for example by avoiding radical surgery and radiotherapy (4, 77).

Stage 4S neuroblastomas without MYCN amplification show spontaneous regression in the majority of patients, and therefore need no or little intervention. However, chemotherapy or low-dose radiotherapy may be indicated for patients with large tumours or massive hepatomegaly with consequently mechanical obstruction, respiratory insufficiency or liver dysfunction (3, 79).

---

**Figure 1:** Risk groups according to the “DCOG NBL 2009 protocol”
Staging according to INSS-staging system. del: delete; imb: imbalance; y: year.

---

**Figure 2:** “DCOG NBL 2009 treatment protocol” for the observation group
Legends: S: surgery; CR: complete remission; HRG: high risk group; MRG: medium risk group
High-risk neuroblastoma

Treatment of high-risk neuroblastomas currently consists of induction, local control, consolidation and maintenance (Figure 4). To improve outcome, it has been shown that dose intense chemotherapy (the same dose in less time given) is better than conventional treatment (80). Local control consists of a combination of surgical resection and external-beam radiotherapy of the primary tumour site. Consolidation aims to eliminate any remaining tumour cells with myeloablative cytotoxic agents and stem cell rescue. Maintenance treatment has been added at the end of treatment protocols with the aim to treat persistent minimal residual disease. Retinoids induce terminal differentiation of neuroblastoma cells in vitro.

The Children’s Oncology Group (COG) reported that immunotherapy with ch14.18 (anti-GD2), GM-CSF, and interleukin-2 was associated with a significantly improved outcome as compared with 13-cis-retinoid alone (event-free survival (EFS) 66% vs. 46% and overall survival (OS) 86% vs. 75% at two years). Future studies are needed to find ways to administer this therapy without severe toxic effects (pain, capillary leak syndrome, hypersensitivity reaction) (81).

131I-MIBG therapy

Neuroblastoma is a radiosensitive tumour and with 90% also being MIBG-avid, these tumours are suitable for targeted 131I-MIBG therapy. This targeted treatment is most effective in patients with a homogeneous uptake of the radiopharmaceutical as the penetration of the bêta-emitter is limited to approximately 5-10 mm. De Kraker et al. reported that 131I-MIBG therapy is effective in patients with newly diagnosed high-risk neuroblastoma with a large tumour mass and high uptake of the radiopharmaceutical
Up to now, toxicity of $^{131}\text{I}$-MIBG has mainly been reported in patients with refractory and relapsed disease that were extensively pre-treated. From these studies it is known that $^{131}\text{I}$-MIBG can cause haematological toxicity with severe and prolonged thrombocytopenia. Toxicity of $^{131}\text{I}$-MIBG as first-line single therapeutic agent has never been reported in a large cohort of patients. Efforts are made to use the proven effective $^{131}\text{I}$-MIBG therapy in consolidation therapy (10). The “DCOG NBL2009 protocol” investigates the use of $^{131}\text{I}$-MIBG therapy as induction treatment.

**Relapses**

More than half of the patients with high-risk neuroblastoma will relapse despite intensive multimodality treatment and an additional 10 to 20% is refractory to induction therapy. Prognosis of these relapsed patients is very poor (83). MYCN amplification, age and time to first relapse were reported as the most important clinical and biological factors that independently predict post-relapse OS in a multivariate analysis of these relapsed and progressing patients (84).

However, cure after relapse is still very rare. Therefore, new approaches to relapsed disease are investigated, such as angiogenesis inhibitors and tyrosine kinase inhibitors. Demethylating agents like decitabine are also under investigation. Furthermore histone deacetylase inhibitors are in clinical trials for patients with refractory solid tumours (4, 77).

**OUTLINE OF THIS THESIS**

MIBG scintigraphy is one of the main diagnostic imaging techniques of neuroblastomas, but there is great variability in the interpretation of diagnostic imaging using MIBG, and other imaging modalities, and uncertainty about the clinical and prognostic consequences of imaging results. Therefore, the first section of this thesis reports on the role of diagnostic imaging in neuroblastoma patients and the importance of the discrimination between bone and bone marrow metastases. In a Cochrane systematic review the diagnostic test accuracy of $^{123}\text{I}$-MIBG scintigraphy and $^{18}\text{F}$-FDG-PET scintigraphy to detect neuroblastoma and its metastases is described (Chapter 2). Since the bone and/or bone marrow metastases are reported as distinct prognostic factors, it is important to differentiate bone from bone marrow involvement when analysing diagnostic images. Consistent reporting and the use of unambiguous nomenclature are warranted. Therefore, a second systematic review reports on the used definitions in the literature of bone and bone marrow metastases in patients with neuroblastoma on diagnostic imaging, and on the diagnostic accuracy of these imaging techniques to detect bone and bone marrow metastases in patients with neuroblastoma (Chapter 3). Furthermore, we compared the ability of $^{123}\text{I}$-MIBG
scintigraphy and MRI-STIR to detect bone and bone marrow metastases in patients with neuroblastoma (Chapter 4).

In metastatic neuroblastoma, two patterns of dissemination have thus far been identified. Stage 4S tumours may show metastases, but these are limited to skin, liver and/or bone marrow. Stage 4 neuroblastoma is defined as all other cases with distant metastatic disease, such as bone metastases, but no separate patterns are described. In the second section, metastatic patterns on $^{123}$I-MIBG scans of stage 4 neuroblastoma patients were determined that correlated with prognostic factors (Chapter 5).

In the third section the role of $^{131}$I-MIBG therapy in unresectable and compromising localised neuroblastoma (Chapter 6) and the toxicity of upfront $^{131}$I-MIBG therapy in newly diagnosed patients (Chapter 7) are reported.
REFERENCES


76. Stichting Kinderoncologie Nederland (SKION), Dutch Childhood Oncology Group (DCOG): DCOG NBL 2009 treatment protocol - for risk adapted treatment of children with neuroblastoma, in , 2009


