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

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Chapter 8

Summary and general discussion
The aims of this thesis were: 1. To investigate the diagnostic accuracy of imaging techniques, 123I-MIBG scintigraphy in particular, to detect neuroblastoma and osteomedullary metastases; 2. To detect patterns of metastases with clinical and prognostic relevance using 123I-MIBG scans; and 3. To investigate the role of 131I-MIBG in the treatment of neuroblastoma.

In this chapter, the main findings of the studies described in the previous chapters will be summarised and discussed. The strengths and limitations of the studies will be discussed and general conclusions will be formulated. Additionally, recommendations for future studies and clinical practice will be given per section.

PART I: METAIODOBENZYL GUANIDINE (MIBG) FOR DIAGNOSTIC PURPOSES

In Part I of this thesis the role of diagnostic imaging, in particular the role of MIBG, to detect neuroblastoma and its metastases was reported.

Objectives:

1. To determine the diagnostic accuracy of 123I-MIBG scintigraphy with or without single photon emission computed tomography (SPECT) - computed tomography (CT) to detect neuroblastoma and its metastases at initial diagnosis and at recurrence (Chapter 2).

2. To determine the diagnostic accuracy of Fluorine-18-fluorodeoxy-glucose (18F-FDG) positron emission tomography (PET), with or without CT to detect neuroblastoma and its metastases at initial diagnosis and at recurrence: solitary, as add-on test if 123I-MIBG scintigraphy was negative and in comparison with 123I-MIBG scintigraphy (Chapter 2).

3. To report all definitions of bone and bone marrow metastases in neuroblastoma used for different imaging techniques and to determine the diagnostic accuracies of these definitions for the different imaging techniques to detect bone and bone marrow metastases (Chapter 3).

4. To compare the ability of 123I-MIBG scintigraphy and magnetic resonance imaging (MRI) - short Ti inversion recovery (STIR) to detect bone and bone marrow metastases in patients with metastatic neuroblastoma and to compare the detected characteristics of the metastases (“focal” or “diffuse”) for both techniques (Chapter 4).

Summary and general discussion

In a diagnostic test accuracy (DTA) Cochrane systematic review of the literature, in Chapter 2, we reported that 123I-MIBG scintigraphy was a sensitive method to detect
neuroblastoma and its metastases with a mean pooled sensitivity of 92.4%. The specificity could not be pooled, because only one study, with a specificity of 85%, reported on false positive findings. In common practice, \(^{123}\)I-MIBG scintigraphy is performed if neuroblastoma is highly suspected, because other diagnostic tests, like ultrasound and urinary catecholamines point towards the tumour. Therefore, false-positive results are rarely reported and the specificity of \(^{123}\)I-MIBG scintigraphy is difficult to assess.

As described in the literature and summarised in Chapter 2, around 10% of neuroblastomas do not accumulate MIBG. For these cases an additional test is needed to detect bone and/or bone marrow metastases. In case of a single equivocal lesion on MIBG scans the international neuroblastoma risk group (INRG) recommends confirmation with another imaging modality, namely, conventional radiography, and MRI or CT if the radiographic findings are negative, or with biopsy (1, 2). Technetium-99 (\(^{99m}\)Tc) bone scintigraphy is usually not required, except in cases in which the primary tumour is not MIBG-avid or MIBG-positivity cannot be confirmed (if the primary tumour is removed or is not MIBG-avid) (1, 2). In case of MIBG-negative neuroblastoma, currently, \(^{18}\)F-FDG-PET-CT is added in international neuroblastoma protocols.

In Chapter 2, a Cochrane systematic review of the literature for the diagnostic accuracy of \(^{18}\)F-FDG-PET-CT was reported and no evidence was found to successfully use this test in addition to \(^{123}\)I-MIBG scintigraphy. However, this test is reported as a promising additional candidate (3). The higher spatial resolution and tomographic nature of the PET technique, in conjunction with the use of FDG and CT, improve disease localisation and detection of small tumours and metastases (4). In contrast to \(^{123}\)I-MIBG imaging, \(^{18}\)F-FDG PET-CT is not specific for neuroblastoma tumours. Furthermore there is a rising concern about cumulative radiation dosage after repetitive \(^{18}\)F-FDG PET-CT investigations during follow-up. \(^{18}\)F-FDG-PET-CT has been reported to be inferior to \(^{123}\)I-MIBG scintigraphy in the detection and follow-up of bone and bone marrow disease (5), while others report it to be superior in monitoring high-risk patients for progressive disease in soft tissue and bone and/or bone marrow, in absence of cranial vault lesions and once the primary tumour was resected (4).

Since the presence of bone and bone marrow metastases are reported as independent adverse prognostic factors for patients with neuroblastoma, we wondered whether these could be differentiated on imaging techniques (6-10). Therefore, we needed definitions of these two entities on imaging techniques. In Chapter 3 we reviewed the literature and concluded that definitions of both bone and bone marrow metastases were not uniformly reported. However, bone metastases on \(^{123}\)I-MIBG scintigraphy were frequently defined as “focal” (11, 12) and bone marrow metastases as “diffuse” (11, 13-16); and on MRI both definitions were used to define bone as well as bone marrow metastases (15, 23-29). The sensitivity and specificity varied widely between the included studies, so no general conclusions could be drawn. The large differences in the reported sensitivity and specificity to detect bone and bone marrow metastases might be explained by the use
of different imaging techniques with their own qualities and pitfalls, different definitions used, and problems with the reference test. For bone metastases a golden standard was not available, because it is not feasible to obtain bone biopsies from each suspect bone lesion in a patient. Since the late 1970s, bone scintigraphy has been the main diagnostic method for the detection of cortical skeletal metastases (30, 31). Therefore it was used in this systematic review as the reference standard to detect bone metastases. With a reported sensitivity and specificity of 70% to 78% and 51%, respectively (32, 33), both false-negative and false-positive results are a problem with using this imaging technique as a reference standard. Nowadays, bone scintigraphy is usually not required, except in cases in which the primary tumour is not MIBG-avid or MIBG-positivity cannot be confirmed (i.e. if the primary tumour has been removed before examination) (1, 2).

Another imaging technique, to detect bone and bone marrow metastases is MRI. This is a highly sensitive imaging technique, but less specific than MIBG scintigraphy, and it contains no ionizing radiation (34, 35). In Chapter 4 we analysed concurrent MIBG and MRI-STIR images of 10 patients and found that MRI-STIR detected more lesions than $^{123}$I-MIBG scintigraphy.

MRI-STIR showed both “focal” and “diffuse” lesions in the bone marrow, so the assumption that focal metastases on MIBG-scintigraphy are bone metastases and diffuse lesions represent bone marrow infiltration, are not correct. Furthermore cortical destruction (representing bone metastases) was accompanied by both “focal” and “diffuse” lesions in the bone marrow on MRI-STIR, and in our small patient sample, these lesions were “diffuse” on MIBG scan.

As “focal” and “diffuse” were both present in the bone marrow on MRI-STIR (Chapter 4) and because it is still not proven that bone and bone marrow metastases are truly different entities (Chapter 3), it might be that all lesions start as small focal lesions in the bone marrow, grow out to diffuse lesions within the bone marrow and finally affect the cortical bone. Therefore, we propose to use the term “osteomedullary” metastases for all skeletal metastatic lesions in neuroblastoma.

Next to the false negative rate of $^{123}$I-MIBG scintigraphy of 10% in Chapter 2, MRI-STIR showed MIBG-non-avid metastases in 28% of all investigated body segments in Chapter 4. Because MIBG is a physiological analogue of norepinephrine (NE), the cellular accumulation of MIBG occurs mainly by active uptake in cells that express the norepinephrine transporter (NET) and only partly by passive diffusion (36). As NET-protein expression correlates with MIBG-avidity and MYCN-amplification is reported to correlate with a lower NET-protein expression in patients with neuroblastoma, MIBG-non-avid lesions might have a different biological background than MIBG-avid lesions.
Strengths and limitations

The quality of the included studies in Chapter 2 and 3 was difficult to assess. Nowadays, thorough reporting of methodology of studies is performed more precisely than in earlier times. Furthermore, nowadays better reference tests to detect bone marrow and bone metastases are available. The methods to detect bone marrow in biopsies/aspirates are more sensitive than in earlier times (37). Bone scintigraphy is currently exclusively used in cases in which the primary tumour is not MIBG-avid or MIBG-positivity cannot be confirmed. More commonly used tests to detect bone metastases are now radiography, CT or MRI, instead of bone scintigraphy (1).

In the Cochrane systematic review, in Chapter 2, only one study could be included that reported on the diagnostic accuracy of 18F-FDG-PET-CT and therefore no conclusions could be drawn on this imaging type.

In the same Chapter 2 we did not include 131I-labelled MIBG scintigraphy, because 123I-MIBG was reported to give superior image quality (3, 38). However, Naranjo et al. reported that both radiopharmaceuticals perform equally the prediction of outcome using a semi-quantitative scoring system (39). Earlier studies frequently contain 131I-MIBG scans and these possibly useful studies were now excluded from the review. Therefore, in the next systematic review on the diagnostic test accuracy to detect bone and bone marrow metastases of neuroblastoma, in Chapter 3, we did include these 131I-MIBG scans.

The studies that were assessed for diagnostic accuracy in Chapter 3, were selected from the studies that were already selected to find definitions of bone and bone marrow metastases. Therefore selection bias cannot be excluded.

In Chapter 4 only ten patients were included, because MRI-STIR was not routinely performed at diagnosis. Furthermore, MRI-STIR was often not performed for the whole-body, but for a body-part, mostly the trunk and proximal extremities, further limiting the available data to study. So, selection bias might have played a role.

We assumed MRI-STIR to detect and differentiate bone from bone marrow metastases. We did not compare the results with a reference test. Ideally, a golden standard for bone as well as bone marrow metastases would be histopathology. All included patients had positive bone marrow biopsies/aspirates. Obtaining histopathological material from all bone lesions in a patient is not feasible. Bone scintigraphy, radiography or CT were not available for most patients (40).

Future perspectives

As reported in Chapter 2, 123I-MIBG scintigraphy might fail to detect neuroblastoma tumours and metastases in around 10% of the cases. These MIBG-non-avid neuroblastomas should be investigated by 18F-FDG-PET-CT and/or MRI. Evidence to use these imaging
techniques to detect neuroblastoma and its metastases should be systematically reviewed, solitary as well as in combination with $^{123}$I-MIBG scintigraphy.

In Chapter 4 we reported that MRI showed more lesions than $^{123}$I-MIBG imaging in patients with stage 4 neuroblastoma. Ideally, a prospective study should be undertaken to investigate the clinical relevance of whole-body MRI-STIR and MIBG imaging at initial diagnosis and during therapy with the following objectives: 1. To investigate whether MIBG imaging combined with bone marrow biopsies/aspirates underestimates the stage of the disease; 2. To investigate whether MIBG-non-avid metastases visible on MRI become MIBG-avid during follow-up and are now defined as progressive or recurrent disease, while in fact these lesions were already present; and 3. To investigate the anatomical localisation of the characteristics of lesions on MIBG imaging by comparing these characteristics (“focal” and “diffuse”) with the characteristics and anatomical findings on MRI.

In the diagnosis of stage MS disease, distinction between bone and bone marrow metastases is important, because metastases are confined to skin, liver, and/or bone marrow with a maximum of 10% bone marrow invasion; moreover bone metastases have to be excluded. The detection of 10% bone marrow invasion is clearly reported (37, 41). However, the detection of bone metastases is not reported, while presence of bone metastases will upstage the neuroblastoma. Therefore, in case of localised MIBG-avid osteomedullary lesions on MIBG scintigraphy, the value of an additional MRI to detect cortical destruction (bone metastases) should be studied.

Recommendations for clinical practice

Because the definitions of bone and bone marrow metastases are unclear and not uniform, no conclusions on diagnostic accuracy could be drawn. To be able to compare results of different trial groups, it is important to use clear and uniform definitions in the future. Therefore, we propose to use the term “osteomedullary” metastases for all skeletal metastatic lesions in neuroblastoma until uniform and unambiguous definitions of bone and bone marrow metastases are found and reported.

As MIBG scintigraphy is a highly sensitive and specific method to detect osteomedullary metastases of neuroblastoma and there is no evidence in the literature yet that other imaging techniques can replace MIBG scintigraphy, it should remain as the first-choice imaging technique in the diagnosis of osteomedullary lesions in patients with neuroblastoma. SPECT-CT enables better depiction of the small focal lesions that are difficult to visualise on planar MIBG scans, especially in areas close to intense physiologic uptake such as the liver and the bladder. However, because of radiation, SPECT-CT should not be added routinely. As recommended by the INRG, one unequivocal MIBG-positive lesion at a distant site is sufficient to define osteomedullary metastases. However, a single
equivocal lesion requires confirmation with conventional radiography and MR imaging or CT if the radiographic findings are negative, or with biopsy. The role of $^{18}$F-FDG-PET-CT and MRI in the detection of osteomedullary metastases in patients with neuroblastoma is still under investigation.

**PART II: $^{123}$I-MIBG AVID METASTATIC PATTERNS IN PATIENTS WITH STAGE 4 NEUROBLASTOMA**

In Part II of this thesis we described metastatic patterns at initial diagnosis in patients with stage 4 neuroblastoma and the clinical relevance of these patterns.

**Objectives**

5. To find clinically relevant MIBG-avid metastatic patterns at initial diagnosis in patients with stage 4 neuroblastoma.

**Summary and general discussion**

In Chapter 5 two MIBG-avid metastatic patterns at initial diagnosis in patients with stage 4 neuroblastoma were reported: a “limited and focal” pattern mainly found in MYCN-amplified neuroblastomas; and an “extensive and diffuse” pattern predominant in MYCN-single-copy neuroblastoma. In a multivariate Cox-regression analysis, focal metastases were associated with a better event-free (EFS) and overall survival (OS) in patients with MYCN-amplification in the COG-cohort than diffuse metastases ($p<0.01$). As there clearly was a difference between focal and diffuse metastases, we wondered what these different characteristics represent. Although the literature does not uniformly report definitions on bone and bone marrow metastases, in the majority of the studies “focal” lesions on $^{123}$I-MIBG scintigraphy are reported to represent bone metastases (11, 12) and “diffuse” lesions bone marrow metastases (11, 13-16) (Chapter 3). In international guidelines for MIBG scintigraphy in children it is reported that skeletal uptake of MIBG can be observed either as focal areas of increased uptake or as diffuse skeletal uptake (43). Furthermore, the SIOPEN scoring-method for MIBG scans distinguishes between focal discrete and more diffuse lesions (44). However, both reports do not give an interpretation to the two types of lesions.

The metastatic patterns that we described suggest that neuroblastomas can metastasise through different biological mechanisms, which might be regulated by MYCN-oncogene activity. MYCN amplification is known to be associated with aggressive behaviour and poor prognosis (45-47). This aggressive behaviour can be explained by involvement of transcriptional targets of MYCN, like cell cycle progression, protein synthesis, metabolism and cell growth. Therefore, one would expect to find more widespread metastases in
MYCN-amplified neuroblastoma. In contrast, we found a preference for MYCN-amplified tumours to present with a “limited and focal” metastatic pattern, whereas MYCN-single-copy neuroblastoma more often had an “extensive and diffuse” pattern. Not all patients with predominantly focal lesions had MYCN-amplification. However, in another study our lab recently described that a subset of MYCN-single-copy neuroblastomas had high MYCN-protein expression. The patients with focal lesions and MYCN-single-copy tumour might have this high MYCN-expression (48).

In the MYCN-transgenic mouse model created by Weiss and co-workers, metastases to the bone marrow with a maximum of 5% invasion were observed (49). Initially, this low rate of metastases to the bone marrow was thought to be model-dependent, but possibly this might not be model dependent, but related with MYCN-overexpression. Granchi et al, reported that in patients with MYCN-single-copy tumours, high Dkk-1 levels gave metastases in three or more sites versus patients with low Dkk-1 with metastases in less than three sites (50). Possibly high Dkk-1 expression correlates with our “diffuse and extensive” metastatic pattern. Previously, our lab showed that DKK1 is downregulated by MYCN, consistent with this observation (51).

As described in Chapter 4, MYCN expression is reported to correlate with a lower NET-protein-expression and in turn NET-protein-expression is correlated with MIBG-avidity. Dubois et al, reported that the median percent NET-protein-expression was 50% (range 0-100%) in MIBG-avid patients compared to 10% (range 0-80%) in non-avid patients (p=0.027). MYCN-amplified tumours had lower NET-protein-expression compared to MYCN-single-copy tumours (10% versus 50%; \( P = 0.0002 \)) (52). Our study showed that patients with MYCN-amplified neuroblastoma did not only present with a predominant focal form of metastases, they also had significant less involved body segments on MIBG imaging at diagnosis. So, one can hypothesise that the lower NET-expression in MYCN-amplified tumours might cause either the focal pattern, or can be involved in MIBG-non-avid lesions with low NET-expression.

Progressive and recurrent events frequently occur in the course of neuroblastoma disease: of the 70% that suffer from events, about 15% do not respond to conventional therapy and never achieve remission; and in about 55% patients suffer from recurrence of the disease (53). Patients with progressive or recurrent neuroblastoma show diversity in the course of the disease, which might be correlated with undiscovered molecular aberrations and they thus respond differently to second-line therapy (54). As two clearly different metastatic patterns of stage 4 neuroblastoma at initial diagnosis had prognostic relevance, these patterns, might act differently upon events and respond differently to treatment. Because recurrent events tend to respond better to second-line treatment and the “focal” pattern had a better outcome in patients with a MYCN-amplified neuroblastoma, focal lesions might be responsible for recurrent events and diffuse lesions for progressive events (55).
Strengths and limitations

In Chapter 5 the two metastatic patterns were found in two separate, but comparable, large cohorts. A limitation was that the European cohort was heterogeneously treated, and therefore could not be investigated for prognostic relevance. This could only be studied in the homogeneously treated Children’s Oncology Group (COG) cohort. Another limitation was that MIBG scans from the European cohort were performed according to different scanning procedures at different centres and that both digital and analogue scans were used. By selecting only high-quality whole-body $^{123}$I-MIBG scans we limited this problem.

Furthermore, the distinction between focal and diffuse metastases was not always clear, especially because sometimes the two characteristics “focal” and “diffuse” co-occurred in one body segment at the same time. However, all $^{123}$I-MIBG scans were evaluated by two observers independently and discordant findings were solved by consensus. Moreover, the metastatic patterns and prognostic analyses were comparable when using single observer scores.

A last limitation might be that we excluded $^{131}$I-MIBG scans, because we aimed to use MIBG scans of comparable quality. Diagnostic $^{131}$I-MIBG scans have been reported to be of lower quality than $^{123}$I-MIBG scans (3). Naranjo et al reported that the scoring of $^{123}$I- and $^{131}$I-MIBG scans had comparable outcomes (39). However, these scoring methods are semi-quantitative, while we are investigating the qualitative characteristics of metastases.

Future perspectives

The two metastatic patterns, especially the characteristics of the lesions, “focal” and “diffuse”, most likely reflect different biological processes. Therefore, ideally both types of metastases on imaging should be correlated with gene expression profiles of these types of metastases. However, it is not feasible to obtain histopathological material from all types of metastases, because it is not feasible to biopsy all lesions in a patient. In the near future it might be possible to select circulating tumour cells from the bone and bone marrow, as this is currently being investigated in a separate PhD project by J. van Wijk (Department of Paediatric Oncology – Academic Medical Centre / Department of Immuno-haematology - Sanquin Research and Landsteiner Laboratory Academic Medical Centre/University of Amsterdam). These gene expression data of circulating tumour cells might represent more correctly the underlying biology of the metastatic lesions than the gene expression data of the primary tumour. If we would select patients with exclusively focal and exclusively diffuse metastases on MIBG imaging, we could investigate whether the gene expression data of circulating tumour cells are different between these two types of metastases. Until this is possible, gene expression data from the primary tumour should be used to correlate with the types of metastases, although one should keep
in mind that metastatic cells might be biologically different from the primary tumour, because during metastatic progression, neuroblastoma cells have undergone a multi-step process. Moreover, neuroblastoma is a very heterogeneous tumour. Therefore, material from one localisation of the primary tumour might contain different gene expression data than the other localisation. By correlating metastatic patterns to gene expression data, eventually new targets for treatment may be found.

To investigate whether the “focal and limited” pattern correlates with a low NET-expression in osteomedullary lesions, retrospectively the patterns should be correlated with NET-expression data, again ideally in circulating tumour cells, but if not available in the primary tumour.

Furthermore, the metastatic patterns found on MIBG scintigraphy should be correlated with MRI to investigate whether there is a correlation between the metastatic pattern observed and the number of MIBG-non-avid lesions. Additionally, MYCN-status should be correlated with the amount of lesions visible on MIBG and on MRI imaging, to investigate whether MIBG-non-avid lesions are more likely to be MYCN-amplified, and to investigate if weak NET-expression, possibly correlated with MYCN-amplification, is responsible for the pattern observed in patients with MYCN-amplified tumours.

A prospective study in a homogeneously treated cohort should confirm the prognostic relevance of the metastatic patterns in patients with stage 4 neuroblastoma, as was seen in the COG-cohort. If the prognostic relevance of the metastatic patterns described in Chapter 5 can be confirmed by a second homogeneously treated cohort, eventually patients with stage 4 neuroblastoma might be subdivided in two risk groups. In addition, in the future it might then be possible to treat patients with different metastatic patterns of stage 4 neuroblastoma according to different treatment protocols that are more targeted at their biological background.

To investigate whether recurrent and progressive events act differently and have a different biological background, in a retrospective study the characteristics of MIBG-avid lesions (“focal” and “diffuse”) on MIBG scans during treatment and at events should be assessed, the origin of lesions (“focal” and “diffuse”) for the events should be scored and their response to treatment should be investigated. As was reported in Part I, at least 17% of osteomedullary metastases in stage 4 neuroblastoma were MIBG-negative and therefore characteristics of lesions detected on 18F-FDG-PET-CT or MRI-STIR should be taken into account as well.

Besides the metastatic patterns concerning characteristics of metastases, patterns might also be present concerning the localisation of the metastases. For example, orbital metastases are uncommon in childhood cancers, but within the very small group of children with orbital metastases, neuroblastomas account for the greater part (56, 57). Furthermore orbital metastases were particularly seen in a subgroup of patients younger than 1.5 years old. It would be interesting to investigate whether clusters of body
segments exist that are more often affected than other clusters of body segments and whether these clusters are prognostically relevant.

PART III: MIBG FOR THERAPEUTIC PURPOSES

In Part III of this thesis the role of $^{131}$I-MIBG therapy in patients with neuroblastoma was reported.

Objectives

6. To investigate the effect of $^{131}$I-MIBG therapy on resectability and outcome in patients with unresectable localised neuroblastoma causing or in danger of causing organ or respiratory dysfunction.

7. To investigate acute toxicity of upfront $^{131}$I-MIBG therapy in a large cohort of neuroblastoma patients (stages 1-4 and 4S).

Summary and general discussion

In Chapter 6, $^{131}$I-MIBG therapy was proved to be an effective treatment modality for unresectable localised neuroblastoma causing or in danger of causing organ or respiratory dysfunction and to offer a good alternative to chemotherapy if urgent treatment was needed.

Since the prognosis of localised neuroblastoma is good, toxicity and late effects should be avoided if possible. We reported a 10-year overall survival (OS) of 90.5 %. Garaventa et al, reported a 5-year OS of 91% for patients with low-risk localised neuroblastoma with standard chemotherapy (vincristine, cyclophosphamide and doxorubicin). In another study Rubie et al. reported a 5-year OS of 99 % for a heterogeneous patient group of 120 infants. This group consisted of patients with and without threatening symptoms and different treatment strategies. The study stated that low-dose chemotherapy without anthracyclines was effective in 62% of infants with an unresectable neuroblastoma with MYCN-single-copy tumours (58). No treatment-related deaths were observed. However, progressive and recurrent events occurred in 12 of 120 patients. In our cohort two patients died. These deaths were not related to $^{131}$I-MIBG therapy but to progression to high-risk disease and to complications of surgery.

Only a few large studies related to unresectable neuroblastoma are reported and conclusions vary. Low-risk localised neuroblastomas were shown to have an excellent prognosis with standard-dose chemotherapy, although other studies reported that these patients with poor prognostic factors did better on a high-dose protocol instead of treatment with a standard-dose protocol (59-61). Poor prognostic factors, like MYCN-amplification, are the cause of most treatment failures (60-62). In our cohort, three patients were treated with $^{131}$I-MIBG and had genetic aberrations that nowadays are considered to account
for high-risk disease. MYCN-amplification and 1p loss of heterozygosity were present in one patient with a stage 3 neuroblastoma. This patient was eventually treated as a high-risk patient after three courses of $^{131}$I-MIBG therapy and was still alive 11 years after treatment. MYCN-amplification was also present in two other patients (stage 2 and 3). These two patients received two courses of $^{131}$I-MIBG therapy followed by a complete resection and are still alive after 12.2 and 7.5 years of follow-up respectively. So, in these cases with poor prognosis no chemotherapy with risk of toxicity was needed.

Although in comparison with chemotherapy, $^{131}$I-MIBG therapy causes little or no toxicity, thyroid toxicity is a known side effect of $^{131}$I-MIBG therapy. Thyroid protection during $^{131}$I-MIBG therapy mostly prevents uptake in the thyroid, but late effects of $^{131}$I-MIBG therapy on thyroid function cannot be ruled out. Recently, two patients from our centre were reported to have differentiated thyroid carcinoma following $^{131}$I-MIBG therapy (63). These patients received adequate thyroid protection during $^{131}$I-MIBG therapy, and no $^{131}$I-MIBG uptake was seen in their thyroid glands on MIBG imaging. So, although no clear evidence is available thus far, patients should be checked regularly for thyroid problems after $^{131}$I-MIBG therapy. None of the patients described in Chapter 6 developed a thyroid carcinoma. Another side effect of $^{131}$I-MIBG can be primary ovarian insufficiency (POI). This is known to be caused by alkylating agents or after radiation to a field that includes the ovaries. Recently two cases of POI, after $^{131}$I-MIBG treatment only, have been described indicating that $^{131}$I-MIBG treatment may have a causative role (64). Lastly, second malignancies have been described in patients that were treated with $^{131}$I-MIBG therapy (65).

Upfront $^{131}$I-MIBG therapy caused only little acute toxicity in patients with newly diagnosed neuroblastoma. In Chapter 7 the most frequently encountered acute toxicity was haematological, followed by nausea and vomiting.

$^{131}$I-MIBG toxicity has predominantly been reported in patients with refractory or relapsed neuroblastoma who have received extensive chemotherapy treatment before $^{131}$I-MIBG therapy. Toxicity of only $^{131}$I-MIBG has therefore been difficult to determine. Haematological toxicity was the main toxicity observed in this pre-treated population and consisted of severe and persistent thrombocytopenia (66, 67). In our cohort of newly diagnosed neuroblastoma patients, grade IV thrombocytopenia occurred in only 1% of patients after the first $^{131}$I-MIBG therapy and in 3% of patients after the second. These percentages are lower than would be expected with induction chemotherapy (68). Mastrangelo et al. also reported only haematological and acceptable toxicity after upfront $^{131}$I-MIBG in combination with chemotherapy (cisplatin, cyclophosphamide, etoposide, vincristine, and doxorubicin) in newly diagnosed neuroblastoma patients (69).

Infections occur frequently after induction chemotherapy due to myelosuppression, and contribute significantly to morbidity and mortality in neuroblastoma patients. Infections have also been reported during and after $^{131}$I-MIBG therapy in heavily pre-treated patients and in patients treated with myelo-ablative $^{131}$I-MIBG therapy (70). In contrast, only a few
patients in our cohort were diagnosed with infections, not exceeding grade II in severity. As patients in our study were chemotherapy-naive, they might have had better bone marrow reserve.

Although upfront $^{131}$I-MIBG therapy in patients with newly diagnosed neuroblastoma patients seemed to be effective without serious toxicity, one should keep in mind that four serious adverse events occurred and in one toxicity of $^{131}$I-MIBG therapy could not be excluded. This patient had a posterior reversible encephalopathy syndrome (PRES), a rare complication in paediatric oncology patients, causing seizures. The PRES was presumably caused by pre-existing hypertension. In the literature only two other cases of PRES have been described in neuroblastoma patients, and none of these patients were treated with $^{131}$I-MIBG (71).

Strengths and limitations

The cohort in Chapter 7 was unique, because patients were treated upfront with $^{131}$I-MIBG therapy before induction therapy was even started. Most studies in the past reported on $^{131}$I-MIBG therapy in refractory neuroblastoma.

A first limitation of both studies is that these were retrospective studies of medical files with subsequently a lot of missing data. One example is that toxicity of $^{131}$I-MIBG therapy is often related to the delivered whole-body dose. Due to the nature of this retrospective study, whole-body doses were not available.

Next, both cohorts were heterogeneous, including patients of all ages (infants and older children), with all stages and different risk profiles. Moreover, the cohort in Chapter 6 was small and included patients that were treated with different pre- en post-treatment modalities additional to the $^{131}$I-MIBG therapy.

Future perspectives

As acute toxicity of upfront $^{131}$I-MIBG therapy was limited if the seriously ill condition of this patient population is taken into consideration, it is now included in the protocol of the Dutch Childhood Oncology Group (DCOG) to investigate whether upfront $^{131}$I-MIBG therapy is feasible in a high-risk treatment protocol compared with the induction chemotherapy regimen of the German Paediatric Oncology Haematology (GPOH) protocol.

Next to acute toxicity it is important to further investigate whether $^{131}$I-MIBG therapy causes long-term toxicity, like thyroid toxicity, primary ovarian insuﬃciency and/or secondary malignancies.

Several studies are using different theoretical approaches to improve the known activity of $^{131}$I-MIBG therapy. The first strategy combines $^{131}$I-MIBG with cytotoxic chemotherapy agents known to also have activity in neuroblastoma. The second strategy involves combining $^{131}$I-MIBG with radiation sensitisers that enhance the anti-tumour effect of
\(^{131}\text{I-}\text{MIBG}\) and may protect normal tissues from damaging effects of radiation. The third strategy involves increasing \(^{131}\text{I-}\text{MIBG}\) uptake by neuroblastoma cells, either by combining \(^{131}\text{I-}\text{MIBG}\) with drugs that increase NET-expression or through the use of high-specific active \(^{131}\text{I-}\text{MIBG}\) (72).

**Recommendations for clinical practice**

Because acute toxicity of \(^{131}\text{I-}\text{MIBG}\) therapy could not be excluded in the case of the serious adverse event with PRES and because hypertensive episodes can occur many hours after \(^{131}\text{I-}\text{MIBG}\) infusion, blood pressure should be monitored for at least 48 hours after administration of \(^{131}\text{I-}\text{MIBG}\).

Infants that are seriously ill or with hypertension are not allowed to have \(^{131}\text{I-}\text{MIBG}\) therapy, because intensive care in radio-protective isolation of these young children is very complicated.

**CONCLUSIONS**

**General conclusions**

1. \(^{123}\text{I-}\text{MIBG}\) scintigraphy is a sensitive method to detect neuroblastoma and its metastases (pooled mean sensitivity of 92.4%, specificity of 85%).
2. There is not enough evidence from the literature to use \(^{18}\text{F-FDG-PET-CT}\) as an additional test.
3. Definitions of bone and bone marrow metastases on imaging techniques are not uniformly reported and the sensitivity and specificity of different imaging techniques to detect bone and bone marrow metastases vary widely, so no general conclusions can be drawn on the diagnostic accuracy to detect bone and bone marrow metastases.
4. “Focal” and “diffuse” metastases on MIBG scans, do not represent bone and bone marrow metastases, as shown on MRI-STIR, but the hypothesis is that metastases are likely to start as small “focal lesions” in the bone marrow, grow out to “diffuse lesions” in the bone marrow and finally affect the cortical bone. Therefore, instead of differentiation between bone and bone marrow metastases, the term “skeletal” or “osteomedullary” metastases might be more appropriate.
5. At initial diagnosis patients with stage 4 neuroblastoma can present with two prognostically relevant MIBG-avid metastatic patterns: a “limited and focal” pattern mainly found in MYCN-amplified neuroblastomas; and an “extensive and diffuse” pattern predominant in MYCN-single-copy neuroblastoma. These patterns suggest that different underlying molecular alterations are involved in the process of metastases.
6. $^{131}$I-MIBG therapy is an effective treatment modality for unresectable localised neuroblastoma causing or in danger of causing organ or respiratory dysfunction and offers a good alternative to chemotherapy if urgent treatment is needed.

7. Upfront $^{131}$I-MIBG therapy causes only little toxicity in patients with newly diagnosed neuroblastoma. The most frequently encountered acute toxicity is haematological, followed by nausea and vomiting. However, because of one serious adverse event with a PRES, toxicity of $^{131}$I-MIBG therapy could not be excluded.

Future perspectives

1. Evidence for the use of $^{18}$F-FDG-PET-CT in addition to $^{123}$I-MIBG scintigraphy should be systematically reviewed in the literature in an update of the DTA Cochrane systematic review.

2. A prospective study should be undertaken to investigate the clinical relevance of whole-body MRI-STIR and MIBG imaging at initial diagnosis and during therapy with the objectives: 1. To investigate whether MIBG imaging combined with bone marrow biopsies/aspirates underestimates the stage of the disease; 2. To investigate whether MIBG-non-avid metastases visible on MRI become MIBG-avid during follow-up and are now defined as progressive or recurrent disease, while in fact these lesions were already present; and 3. To investigate the anatomical location of the characteristics of lesions on MIBG imaging by comparing these characteristics (“focal” and “diffuse”) with the characteristics and anatomical findings on MRI.

3. In case of localised MIBG-avid osteomedullary lesions on MIBG scintigraphy in patients suspected of stage 4S neuroblastoma, the value of an additional MRI to detect cortical destruction (bone metastases) should be studied.

4. The two metastatic patterns, especially the characteristics of the lesions, focal and diffuse, should be correlated with gene expression profiles. Ideally gene expression data of isolated neuroblastoma cells from the bone and/or bone marrow should be used. Until this is possible, gene expression data from the primary tumour can be used.

5. To investigate whether the “focal and limited” pattern correlates with a low NET-expression in osteomedullary lesions, retrospectively the patterns should be correlated with NET-expression data, ideally in circulating tumour cells, but if not available in the primary tumour.

6. The metastatic patterns found on MIBG scintigraphy should be correlated with MRI to investigate whether there is a correlation between the metastatic pattern observed and the number of MIBG-non-avid lesions. Additionally, MYCN-status should be correlated with the amount of lesions visible on MIBG and on MRI imaging, to investigate whether MIBG-non-avid lesions are more likely to be MYCN-amplified, and to investigate if weak NET-expression, possibly correlated with MYCN-amplification, is responsible for the pattern observed in patients with MYCN-amplified tumours.
7. A prospective study in a homogenously treated cohort should confirm the prognostic relevance of the metastatic patterns at initial diagnosis in patients with stage 4 neuroblastoma, as was seen in the COG-cohort. If the prognostic relevance of the metastatic patterns can be confirmed by a second homogeneously treated cohort, eventually patients with stage 4 neuroblastoma might be subdivided in two risk groups. In addition, in the future it might then be possible to treat patients with different metastatic patterns according to different treatment protocols that are more targeted at their biological background.

8. To investigate whether recurrent and progressive events act differently and have a different biological background, in a retrospective study the characteristics of MIBG-avid lesions (“focal” and “diffuse”) on MIBG scans during treatment and at events should be assessed, and their origin and their response to treatment should be investigated. Also, characteristics of lesions detected on $^{18}$F-FDG-PET-CT or MRI-STIR should be taken into account.

9. Next to acute toxicity it is important to investigate whether $^{131}$I-MIBG therapy causes long-term toxicity, like thyroid toxicity, gonadal toxicity and secondary malignancies.

10. To improve the known activity of $^{131}$I-MIBG therapy in patients its combination with the following should be investigated: cytotoxic chemotherapy agents known to also have activity in neuroblastoma, with radiation sensitisers that enhance the anti-tumour effect of $^{131}$I-MIBG and may protect normal tissues from damaging effects of radiation, and with drugs that increase NET-expression.

Implications for clinical practice

1. The term “osteomedullary” metastases for all skeletal metastatic lesions in neuroblastoma should be used until uniform and unambiguous definitions of bone and bone marrow metastases are found and reported.

2. MIBG scintigraphy should remain the imaging technique of first choice in the diagnosis of osteomedullary lesions in patients with neuroblastoma. The role of $^{18}$F-FDG-PET-CT and MRI in the detection of osteomedullary metastases in patients with neuroblastoma is still under investigation.

3. Infants that are seriously ill or have hypertension are not allowed to have $^{131}$I-MIBG therapy, because intensive care during radio-protective isolation of these young children is very complicated. Blood pressure should be monitored for at least 48 hours after administration of $^{131}$I-MIBG in all patients.
REFERENCES


