

Evaluation of the SIOOPEN semi-quantitative scoring system in planar simpatico-adrenal MIBG scintigraphy in children with neuroblastoma

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Neuroblastoma is the most common malignancy in children comprising 7.6% of all infantile cancers. MIBG scintigraphy is a mandatory neuroblastoma diagnostic test, which is among others methods, semi-quantified by the SIOOPEN method. The aim of this study was to test both the skeletal and the soft tissue segments of the SIOOPEN scoring method in the diagnostic milieu and to correlate them with the Curie score. Since there is little knowledge of their diagnostic power, the following variables were tested: VMA, HVA, LDH, and MYCN, ferritin, bone marrow infiltration, the INSS and the INPC classification. The cross-sectional study with repeated measurements of 143 scintigrams was performed on 76 pediatric patients with suspected or proven neuroblastoma, who had been referred to the Center for Nuclear Medicine of the Clinical Center of Serbia in the period 2007-2012. The range of the SIOOPEN soft tissue scores was 0-5. The range of the SIOOPEN skeletal scores was 0-57. The range of the Curie scores was 0-26. The skeletal SIOOPEN scores were significantly higher in bone marrow positive children, in children with pathologically elevated urinary VMA levels and in children having a more advanced clinical stage. There was no difference in the SIOOPEN soft tissue score due to higher VMA levels, or depending on the clinical stage and positive bone marrow assessment. There was no difference between the SIOOPEN skeletal and soft tissue scores on one hand and the histological grade of the tumor; elevated or normal levels of HVA, LDH, NSE and ferritin, or the presence or absence of MYCN amplification in the neuroblastoma cell line, on the other hand. The results of both SIOOPEN scores showed a high linear correlation with the Curie score. The conclusion is that the soft tissue segment of the SIOOPEN score needs further elucidation in a more controlled milieu. Excellent correlation between all segments of the two semi-quantitative scoring methods speaks in favor of the application of the complete SIOOPEN scoring system in every day mIBG scanning.

Key words: neuroblastoma, mIBG scintigraphy, MYCN amplification, catecholamine, semi-quantitative, score

Neuroblastoma is the most common malignancy in children comprising 7.6% of all infantile cancers. The median patient age is 15 months, with the survival rate of 59% to 66%

[1]. This tumor of variable biological and clinical behavior secretes catecholamines which are readily metabolized into vanillylmandelic (VMA) and homovanillic acid (HVA), the most sensitive and specific neuroblastoma tumor markers. It produces neuron-specific enolase (NSE), lactate dehydrogenase (LDH), as well as ferritin. One of its prognostic hallmarks is MYCN gene amplification. Neuroblastoma is diagnosed on the basis of the unequivocal presence of neuroblasts in tumor tissue or on the basis of the presence of neuroblasts in bone marrow and increased urinary or serum catecholamine levels. The tumor is clinically staged according to the International Neuroblastoma Staging System (INSS). Its histology grade is

List of abbreviations: EANM – European Association of Nuclear Medicine; EFS – event – free survival; FISH – Fluorescence in situ hybridisation; HVA – homovanillic acid; INPC – International Neuroblastoma Pathology Classification; INRGSS – International Neuroblastoma Risk Group Staging System; INSS – International Neuroblastoma Staging System; LDH – lactate dehydrogenase; mIBG – meta-iodobenzylguanidine; MYCN – neuroblastoma MYC oncogene; NSE – neuron-specific enolase; S.D. – standard deviation; SIOOPEN – International Society of Paediatric Oncology Europe Neuroblastoma Group; VMA – vanillylmandelic acid

determined by the International Neuroblastoma Pathology Classification System (INPC).

Viable neuroblasts accumulate metaiodobenzylguanidine (mIBG), an aralkyl-guanidine analogue of catecholamine precursors. MIBG scintigraphy has a sensitivity of 88%-93% and specificity of 83%-92% in neuroblastoma which is why it is incorporated as a mandatory test in the International Neuroblastoma Risk Group Staging System (INRGSS), an imaging defined staging and risk assessment system. [2, 3]

In order to facilitate the comparison of mIBG scans and to increase their reliability and reproducibility semi-quantitative scoring systems have been developed. The Curie method [4] has been the most evaluated one, in the sense of prognosis and treatment evaluation, until the introduction of the SIOOPEN (International Society of Pediatric Oncology Europe Neuroblastoma Group) method. The SIOOPEN scoring method is currently under prospective evaluation in Europe. However, as far as the authors of this study are aware, only its skeletal segment has been assessed, although it has been shown that failing to assess soft tissue lesions could result in an inaccurate response evaluation. [5-7] This is why in this study an attempt has been made to test both the skeletal and the soft tissue segment of the SIOOPEN scoring method in the diagnostic milieu and to correlate them with the Curie score. Since there is little evaluation of their diagnostic power, the following variables were chosen to be tested: VMA, HVA, LDH, MYCN, ferritin, bone marrow infiltration, the INSS and the INPC classification. [8] These variables were the most accessible ones in our study group.

Materials and methods

76 pediatric patients with neuroblastoma, who had been referred to the Centre for Nuclear Medicine of the Clinical Centre of Serbia for I-123/131 MIBG scintigraphy from January 2007 to December 2012, were investigated in this study. The cross-sectional study with repeated measurements of 143 scintigrams included 39 girls and 37 boys of the mean age 31.9 months at the first scintigraphy (range: 0–135 months) performed at the Center for Nuclear Medicine. In line with international agreement, patients were referred to the Center by pediatricians because of suspected or proven neuroblastoma. They were sent for an MIBG scan for initial diagnosis or for evaluation after treatment.

I-123 MIBG scintigraphy. I-123/131 MIBG scintigraphy was performed according to previously published guidelines; the images were acquired 24 h or 48 h after slow intravenous injection of no less than 80 MBq (I-123) or 35 MBq (I-131) in accordance with the EANM (European Association of Nuclear Medicine) dosage card. The imaging was conducted using single or dual-head gamma cameras equipped with a high-energy or low-energy collimator (Digitrac 37, Siemens; Nucline™ Spirit, Mediso; ECAM, Siemens). Whole-body planar images were acquired at scanning speeds of 6cm/min if I-123 MIBG was employed. If I-131-MIBG was used, spot images of 250,000 counts per view or 10 minute acquisition time were applied [9, 10]

Image interpretation and scoring. For every patient, the I-123 MIBG and I-131 MIBG scans were scored by the Curie and the SIOOPEN methods. The SIOOPEN method included both the skeletal (osteo-medullary) and the soft tissue score. The scoring was performed as follows. According to the Curie semi-quantitative scoring method, the skeleton was divided into nine areas, and a tenth sector counting any soft tissue involvement was added. These areas correspond to: the head and the face (area 1) the neck and back vertebral column (area 2), the ribs and the sternum (area 3), the lumbar and sacral column (area 4), the pelvis (area 5), the arms (area 6), the fore-arms and the hands (area 7), the thighs (area 8), the legs and the feet (area 9). In each region, the number of lesions was quoted as follows: 0, no site per segment; 1, one site per segment; 2, more than one site per segment; 3, massive involvement (>50% of the segment area). The overall absolute scores were obtained by adding the scores corresponding to each region (maximum 30).

According to the SIOOPEN semi-quantitative scoring method, the skeleton was divided into 12 anatomical body segments as follows: the skull, the thoracic cage, the proximal right upper limb, the distal right upper limb, the proximal left upper limb, the distal left upper limb, the spine, the pelvis, the proximal right lower limb, the distal right lower limb, the proximal left lower limb and the distal left lower limb. The extent and pattern of skeletal mIBG involvement was scored using a 0–6 scale to discriminate between focal discrete lesions and patterns of more diffuse infiltration. Each segment was scored as 0, no involvement; 1, one discrete lesion; 2, two discrete lesions; 3, three discrete lesions; 4 more than 3 discrete foci or a single diffuse lesion involving 50% of a bone; 5, diffuse involvement of 50% to 95% of the entire bone; 6, diffuse involvement of the entire bone, with a maximum score of 72. The soft tissue SIOOPEN score was calculated in the regions of the head and neck, the thorax, the right upper limb, the left upper limb, the abdomen, the pelvis, the right lower limb and the left lower limb. Each segment was scored as 0: no involvement; 1: one solitary lesion and 2: multiple lesions. [7, 11]

Statistical analysis. The concurrence of the SIOOPEN scores with biological and diagnostic tumor features (VMA, HVA, LDH, NSE, ferritin, bone marrow assessment, the INSS and the INPC) were tested by the Mann-Whitney U test, the Kruskal-Wallis chi-square test and the Spearman r correlation coefficient. The correlation between the SIOOPEN scores and the Curie score was established by the Spearman r correlation coefficient. All statistical tests were two-sided and a p value < 0.05 was considered statistically significant.

This investigation was approved by the Institutional Review Board of the hospital. MIBG scans were acquired with the informed consent of the parents.

Results

Patients' characteristics. The clinical and histological stages of the patient population are shown in Table 1. There

were 8 patients in stage 1, 4 patients in stage 2, 10 patients in stage 3 and 40 patients in stage 4 at the time of the first mIBG scan performed for the purpose of the study.

51 patients out of 76 were histologically classified as follows: 10 undifferentiated neuroblastomas with unfavorable histology; 13 poorly differentiating neuroblastomas with unfavorable histology; 16 differentiating neuroblastomas with favorable histology; 5 ganglioneuroblastomas; 2 ganglioneuroblastomas, nodular; 5 neuroblastomas, unclassified. (Table 1)

Biochemical findings. In our study group, VMA was found to be positive in 43 out of 63 tested patients at the time of the first scintigraphy; HVA was positive in 32 out of 48 patients; MYCN in 7 out of 27; LDH in 28 out of 36 tested patients; NSE in 24 out of 26; ferritin in 16 out of 32, and bone marrow was infiltrated in 12 out of 39 tested patients.

Scintigraphic findings. All 76 children had at least one scintigraphy. Out of the 76 subjects, 31 children had 2 scintigraphies, 20 children had 3 scans, 8 children had 4 scans, 5 children had 5 scans, 2 children had 6 and one child had 7 scintigraphies. The median age at the second and further scans was: 38, 46.5, 68, 81,158 and 150 months, respectively. The SIOPEN soft tissue scores ranged between 0-5. The SIOPEN skeletal scores ranged between 0-57. The Curie scores ranged between 0-26.

The skeletal SIOPEN scores were significantly higher in bone marrow positive children (Mann-Whitney U=77.9; p=0.009), in children with pathologically elevated urinary VMA levels (Mann-Whitney U=322; p=0.038) and in children in a more advanced clinical stage (Spearman r=0.37, p=0.003). The SIOPEN soft tissue score showed no difference in relation to the aforementioned higher VMA levels, more advanced clinical stage and positive bone marrow assessment. (Table 2) There was no difference between the SIOPEN skeletal and soft tissue scores on one hand and the histological tumor grade; elevated or normal levels of HVA, LDH, NSE and ferritin; and the presence or absence of MYNC amplification in the neuroblastoma cell line, on the other hand.

Table 1. Clinical and histology stage of patients presented at the first scintigraphy.

INSS	N
1	8
2	4
3	10
4	40
4s	1
histology grade	
undifferentiating unfavourable	10
poorly differentiating unfavourable	13
differentiating favourable	16
ganglioneuroblastoma	5
ganglioneuroblastoma, nodular	2
neuroblastoma non classified	5

The results of both SIOPEN scores and the Curie score showed a high linear correlation at the first three examination time points, i.e. MIBG scans. (At the first scan (n=75): SIOPEN soft tissue, r = 0.696; SIOPEN skeletal, r = 0.676; at the second

Table2. Distribution of the SIOPEN scores by biological/diagnostic features of neuroblastoma

	SIOPEN score, soft tissue	SIOPEN score, bone
Bone marrow biopsy		
Positive		
mean ± S.D.	0.58 ± 0.79	18.08 ± 20.38*
median (range)	0 (0 – 2)	0 (0 – 55)
Negative		
mean ± S.D.	0.89 ± 0.89	1.44 ± 7.31
median (range)	1 (0 – 3)	0 (0 – 38)
VMA		
Positive		
mean ± S.D.	0.53 ± 0.735	8.74 ± 16.16*
median (range)	0 (0 – 2)	0 (0 – 57)
Negative		
mean ± S.D.	1.0 ± 1.26	0.15 ± 0.5
median (range)	1 (0 – 4)	0 (0 – 2)
HVA		
Positive		
mean ± S.D.	0.56 ± 0.8	8.97 ± 17.35
median (range)	0 (0 – 2)	0 (0 – 57)
Negative		
mean ± S.D.	0.81 ± 1.17	1.13 ± 4.24
median (range)	0 (0 – 3)	0 (0 – 17)
MYCN		
Positive		
mean ± S.D.	0.86 ± 0.69	6.29 ± 11.93
median (range)	1 (0 – 2)	0 (0 – 31)
Negative		
mean ± S.D.	0.65 ± 0.93	4.2 ± 13.28
median (range)	0 (0 – 3)	0 (0 – 55)
Ferritin		
Positive		
mean ± S.D.	0.94 ± 0.85	8.69 ± 15.75
median (range)	1 (0 – 4)	0 (0 – 55)
Negative		
mean ± S.D.	1.0 ± 1.55	8.06 ± 18.26
median (range)	1 (0 – 4)	0 (0 – 57)
LDH		
Positive		
mean ± S.D.	0.75 ± 1.00	10.11 ± 18.17
median (range)	0.5 (0 – 4)	0 (0 – 57)
Negative		
mean ± S.D.	0.75 ± 0.71	0 ± 0
median (range)	1 (0 – 2)	0 (0 – 0)
NSE		
Positive		
mean ± S.D.	0.63 ± 0.77	6.54 ± 15.99
median (range)	0 (0 – 2)	0 (0 – 55)
Negative		
mean ± S.D.	1.0 ± 0	0.5 ± 0.71
median (range)	1 (1 – 1)	(0 – 1)

*Statistically significance (p<0.05)

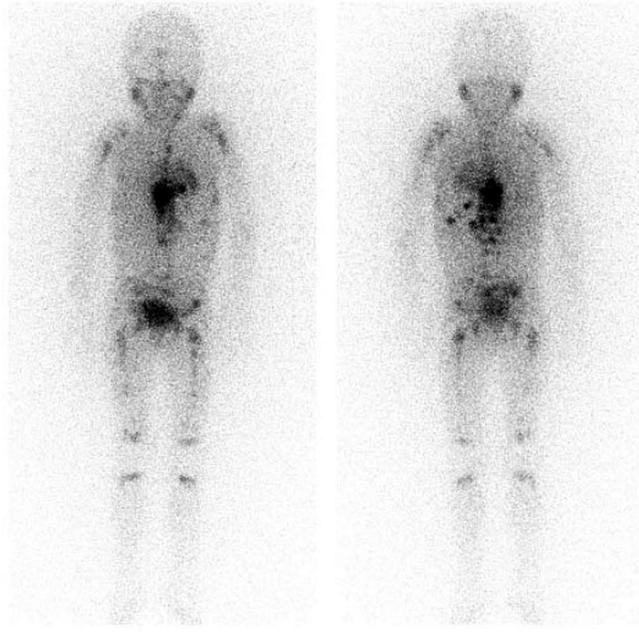


Figure 1. Scintigraphy scoring according to both methods in a 49-month-old boy diagnosed as poorly differentiating stage 4 neuroblastoma, MYCN negative, but with 17q gain; SIOPEN skeletal score 33 (skull and facial bones 0; thoracic cage 4; right humerus 3; left humerus 3; right forearm 0; left forearm 0; spine 4; pelvis 3; right femur 4; left femur 4; right tibia/fibula 4; left tibia/fibula 4). SIOPEN soft tissue score 4 (head and neck 0; thorax 2; right upper limb 0; left upper limb 0; abdomen 2; pelvis 0; right lower limb 0; left lower limb 0). CURIE score 13: head and face 0; neck and back vertebral column 2; ribs and the sternum 0; lumbar and sacral 501 column 0; pelvis 2; arms 2; fore-arms and the hands 0; thighs 2; legs and feet 2; soft tissue sector 3.

scan (n=31): SIOPEN soft tissue, $r = 0.715$ SIOPEN skeletal, $r = 0.47$ (all $p < .001$); at the third scan (n=20): SIOPEN soft tissue, $r = 0.509$ ($p = 0.22$) SIOPEN skeletal, $r = 0.656$ ($p = 0.002$). (Table 3)

Figure 1 shows scintigraphy scoring according to both methods in a 49-month-old boy diagnosed as poorly differentiating stage 4 neuroblastoma, MYCN negative, but with 17q gain. The SIOPEN method yielded the soft tissue score of 4 and the skeletal score of 33. The Curie score was 13.

Table 3. Correlation between Curie score and the SIOPEN scores

Curie score	n	SIOPEN soft tissue score r	p	SIOPEN bone score r	p
1 st scintigraphy	75	0.691	<0.001	0.676	<0.001
2 nd scintigraphy	31	0.715	<0.001	0.470	0.008
3 rd scintigraphy	20	0.509	0.022	0.656	0.002
4 th scintigraphy	7	0.491	0.263	0.676	0.096
5 th scintigraphy	3	0.866	0.333	0.866	0.333

Discussion

The study of the evaluation of neuroblastoma diagnostic and staging markers, with the correlation of both SIOPEN scores with the Curie score, led to several findings. Firstly, the SIOPEN skeletal score was significantly higher in children with pathologically elevated urinary VMA levels, a more advanced clinical stage and bone marrow metastases. On the other hand, the SIOPEN soft tissue score did not show a correlation with VMA levels and the clinical stage of the disease. Neither SIOPEN score concurred with the histological tumor grade, VMA, LDH, NSE, ferritin levels and MYCN amplification. Finally, this study showed a high correlation between both SIOPEN scores and the Curie score.

An elevated serum level of VMA, found 16% less frequently than an elevated urine level in neuroblastoma, has previously shown to be useful as a tumor marker in localized disease but not in metastatic neuroblastoma. Our data differs somewhat from this, suggesting that urinary VMA positive patients had higher scores in the metastatic osteo-medullary segment. An elevated VMA level, which is detected in up to 60% of metastatic patients, is also seen to significantly follow mIBG changes. However, the mIBG scan is considered a better monitoring tool for disease extent evaluation. Therefore, we speculated that augmented levels of urinary catecholamines may be found in children with poorly differentiated neuroblastoma, meaning that less differentiated neuroblastoma is more prone to metastasize in bone tissue, yielding higher SIOPEN skeletal scores in urinary VMA positive patients and in patients in a more advanced clinical stage. The correlation between the tumor stage and the VMA was reported since patients in a less advanced stage or with a localized disease were more likely to have a negative urinary VMA result. This was expected because the highest clinical INSS tumor grade indicates the presence of osteo-medullary metastases, in addition to others, and the majority of the subjects in the group examined in the present study were patients with stage 4 neuroblastoma (40 of 76).

The predominance of stage 4 neuroblastoma in this study may be explained in several ways. Firstly, the incidence of stage 4 neuroblastoma at diagnosis is the highest among all tumor INSS stages. Secondly, the introduction of mIBG imaging enhanced the ability of detecting metastatic disease. As a sensitive and fairly expensive diagnostic tool, mIBG scanning in Serbia is, for the time being, available for preselected cases (e.g. children are diagnosed in tertiary referral centers, where they are admitted most often in an advanced stage of neuroblastoma). [2, 12-14]

A significant positive correlation was found between the clinical INSS tumor stage and the skeletal score but this was not the case with the soft tissue score. The studied group comprised all five INSS clinical stages (only one patient in the group was staged 4s), which differs from similar research conducted in more homogenous samples. The majority of the subjects in the group were stage 4 neuroblastoma patients (40 out of 76) which is why a positive correlation between the clinical INSS tumor stage and

the skeletal score was somewhat to be expected. The lack of correlation between the soft tissue SIOPEN score and the INSS stage could, therefore, be explained by a small number of patients with metastases located in other site than in the skeleton.

The median age of our patients was 31.5 months, which is older the median age of 15 months reported by Kaatsch. The explanation for this lies in the fact that children in Serbia are presented for the first scintigraphy mostly after the diagnosis of neuroblastoma has already been established, i.e. for a follow up after treatments. There was a slightly greater number of girls than boys.

The SIOPEN skeletal scores were significantly higher in patients with a positive bone marrow biopsy. The authors of the present study found that this significant concurrence between bone marrow infiltration and the SIOPEN skeletal score was similar to a quite high, but not absolute correlation between mIBG findings and bone marrow findings, at the same site, found by Frappaz et al. The results of the present study are additionally supported by the fact that the I-123-mIBG scan finding has a high specificity for detecting bone marrow invasion and a very high sensitivity in excluding it; it also has a greater sensitivity than the conventional cytological examination of routinely obtained bone marrow smears from the iliac crest. [15-24]

We found a similar frequency of MYCN amplified patients in our group to the one reported by Ihara et al. [25] The SIOPEN scores were no different in the sense of amplified or non-amplified MYCN patients. It has been stated that the MYCN amplified gene is a reliable indicator of an unfavorable prognosis but its clinical role is controversial and as yet not clarified. MYCN amplification determined by the FISH (fluorescence in situ hybridization) method is believed to be strongly and inversely correlated with the prognosis, therefore it can be correlated in a similar manner with the SIOPEN scores, especially with the skeletal one. No difference was found between either of the SIOPEN scores and MYCN amplification. No conclusion could be drawn from this finding. The result could be the consequence of a small number of tested and a small number of MYCN amplified patients (7 positive out of 27 tested) in a study group that included patients in all clinical stages, or the fact that the gene has only a prognostic significance, although some researchers have reported no significant association between MYCN amplification and the overall and progression-free survival, in a relatively homogenous cohort that included only stage 4 neuroblastoma patients. [26, 27]

The same was assumed for elevated NSE levels on the basis of a significant correlation of this marker and the clinical stage, as well as its greater incidence in metastatic disease especially in the 4s stage. Also, NSE in contrast to LDH was found to be far better in detecting metastatic recurrence. On the other hand, LDH was an independent prognostic factor only in stage 4 patients without MYCN amplification, and ferritin levels were positively associated with the stage of the disease, without having a predictive power. [28-30] The lack of correlation between the SIOPEN scores and NSE, LDH and

ferritin was attributed to the heterogeneity of the sample and the small number of patients with low-grade neuroblastoma, this with the knowledge that these markers can better depict localized than metastatic disease.

The histological ICPN tumor grade also showed no difference between the two SIOPEN scores. There are no data in literature, as far as the authors of this study know, that significantly links the mIBG scan and the histology grade of neuroblastoma.

Excellent correlation between both SIOPEN scores and the Curie score was somewhat expected, bearing in mind their previously well-established equality in outcome prediction and EFS (event-free survival). A recently reported study on 71 neuroblastoma patients in clinical stage 4, which compared the Curie score and the SIOPEN skeletal score with respect to outcome, did not point out either of the scores as a superior one. However it clearly stated their prognostic significance and demonstrated excellent inter-observer reliability of both. This study applied the Curie score, which includes extra-osseous metastases (soft tissue involvement score), and the SIOPEN skeletal score, which assesses osteo-medullary metastases only. [31] Therefore it is not surprising that the SIOPEN soft tissue score had a strong positive correlation with the Curie score in the present study.

Study limitation. The results were based on the analysis of a relatively small sample without age stratification. Both the I-123 and I-131 mIBG scans were included (54 vs. 89) with the authors fully aware of the fact that the I-123 mIBG Curie score has a proven greater sensitivity and that risk of higher radiation of I-131mIBG is lesser than the risk of not performing a mIBG scan at all. However, Naranjo et al. reported no difference in post induction survival evaluated by the Curie mIBG score using both I-123 and I-131mIBG scans [32]. The biological markers of neuroblastoma (urinary VMA and HVA, NSE, LDH, ferritin, MYCN) and bone marrow biopsy were not acquired in all, but in the majority of the patients. Only several patients were presented for initial mIBG scintigraphy. The majority of the patients were mIBG tested after surgical treatment or chemotherapy (there were 34 pre therapeutic soft tissue scores: 12 scored 0 and the majority of 15 (44.7%) scored 1). This consequently led to few patients with metastases located somewhere other than in the skeleton and therefore to a lack of correlation between the soft tissue SIOPEN score and the INSS clinical stage. This is somewhat supported by Du Bois who, in a group of 434 stage 4 neuroblastoma children, demonstrated predominant osteo-medullary metastases in the older patients (> 1 year) [33].

In conclusion, in spite of these limitations, the data from this study clearly demonstrated once more the reliability of the SIOPEN scoring method in detecting skeletal involvement in patients with elevated VMA levels and bone marrow infiltration. The soft tissue segment of the SIOPEN score needs further elucidation in a more controlled milieu. Excellent correlation between all parts of the two semi-quantitative scoring methods speak in favor of the application of the complete SIOPEN scoring system in everyday mIBG scanning.

References

- [1] KAATSCH P Epidemiology of childhood cancer. *Cancer Treat Rev* 2010; 36: 277–85. <http://dx.doi.org/10.1016/j.ctrv.2010.02.003>
- [2] BERTHOLD F, SIMON T Clinical presentation, In: Cheung NK, Cohn S, editors. *Neuroblastoma*, Springer – Verlag Berlin Heidelberg 2005; 63–85
- [3] SHIMADA H, NAKAGAWA A Pathology of the Peripheral Neuroblastic Tumors. *Lab Med* 2006; 37: 684–689. <http://dx.doi.org/10.1309/0506C1BM8GBVV224>
- [4] ADY N, ZUCKER JM, ASSELAIN B, EDELINE V, BONNIN F et al. A new 123I – MIBG whole body scan scoring method – Application to the prediction of the response of metastases to induction chemotherapy in stage IV neuroblastoma. *Eur J Cancer* 1995; 31A: 256–261. [http://dx.doi.org/10.1016/0959-8049\(94\)00509-4](http://dx.doi.org/10.1016/0959-8049(94)00509-4)
- [5] MESSINA JA, CHENG SC, FRANC BL, CHARRON M, SHULKIN B et al. Evaluation of semi-quantitative scoring system for metaiodobenzylguanidine (mIBG) scans in patients with relapsed neuroblastoma. *Pediatr Blood Cancer* 2006; 47: 865–74. <http://dx.doi.org/10.1002/psc.20777>
- [6] LEWINGTON V, BAR-SEVER Z, LYNCH T, MCEWAN AJB, GIAMMARILE F et al. Development of a semi-quantitative mIBG reporting method system. *Pediatr Blood Cancer* 2011; 53: 808
- [7] HRNBL1-ESIOP_protocol_anglais_amendement4_juillet2009.pdf http://www.oncavergne.fr/index.php?option=com_docman&task=doc_download&gid=928&Itemid
- [8] RILEY RD, HENEY D, JONES DR, SUTTON AJ, LAMBERT PC et al. A systematic review of molecular and biological tumor markers in neuroblastoma. *Clin Cancer Res* 2004; 10: 4–12. <http://dx.doi.org/10.1158/1078-0432.CCR-1051-2>
- [9] OLIVIER P, COLARINHA P, FETTICH J, FISCHER S, FROKIER J et al. Guidelines for radioiodinated MIBG scintigraphy in children. *Eur J Nucl Med Mol Imaging* 2003; 30: B45–50. <http://dx.doi.org/10.1007/s00259-003-1138-9>
- [10] <http://www.eanm.org/docs/dosagecard.pdf>
- [11] MATTHAY KK., SHULKIN B, LADENSTEIN R, MICHON J, GIAMMARILE F et al. Criteria for evaluation of disease extent by 123I-metaiodobenzylguanidine scans in neuroblastoma: a report for the International Neuroblastoma Risk Group (INRG) Task Force. *B J Cancer* 2010; 102: 1319–1326. <http://dx.doi.org/10.1038/sj.bjc.6605621>
- [12] SCHROEDER H, WACHER J, LARSSON H, ROSTHOEJ S, RECHNITZER C, PETERSEN BL et al. Unchanged incidence and increased survival in children with neuroblastoma in Denmark 1981–2000: a population-based study. *Br J Cancer* 2009; 100: 853–7. <http://dx.doi.org/10.1038/sj.bjc.6604922>
- [13] BERNSTEIN ML, LECLERC JM, BUNIN G, BRISSON L, ROBISON L et al. A population-based study of neuroblastoma incidence, survival, and mortality in North America. *J Clin Oncol* 1992; 10: 323–329)
- [14] NAVALKELE P, O'DORISIO MS, O'DORISIO TM, ZAMBA GK, LYNCH CF Incidence, survival, and prevalence of neuroendocrine tumors versus neuroblastoma in children and young adults: nine standard SEER registries, 1975–2006. *Pediatr Blood Cancer* 2011; 56: 50–57. <http://dx.doi.org/10.1002/psc.22559>
- [15] PEREL Y, CONWAY J, KLETZEL M, GOLDMAN J, WEISS S et al. Clinical impact and prognostic value of metaiodobenzylguanidine imaging in children with metastatic neuroblastoma. *Pediatr Hematol Oncol* 1999 Jan-Feb; 21: 13–8. <http://dx.doi.org/10.1097/00043426-199901000-00004>
- [16] FRAPPAZ D, COMBARET V, DESUZINGES C, BOUFFET E, BAILLY C et al. Can MIBG scan replace the need for bone marrow assessment at diagnosis and reassessment in stage 4 neuroblastomas? *Bull Cancer* 2004; 91: E253–60.
- [17] ERDELYI DJ, ELLIOT M, PHILLIPS B Urine catecholamines in pediatrics. *Arch Dis Child Educ Pract Ed* 2011; 96: 107–111. <http://dx.doi.org/10.1136/adc.2010.207126>
- [18] BERTHOLD F, HUNNEMAN DH, HARMS D, KÄSER H, ZIESCHANG J Serum vanillylmandelic acid/homovanillic acid contributes to prognosis estimation in patients with localized but not with metastatic neuroblastoma. *Eur J Cancer* 1992; 28: 1950–1954. [http://dx.doi.org/10.1016/0959-8049\(92\)90234-S](http://dx.doi.org/10.1016/0959-8049(92)90234-S)
- [19] SIMON T, HERO B, HINNEMAND H, BERTHOLD F Tumor marker are poor predictors for relapse or progression in neuroblastoma. *Eur J Cancer* 2003; 39: 1899–1903 [http://dx.doi.org/10.1016/S0959-8049\(03\)00376-9](http://dx.doi.org/10.1016/S0959-8049(03)00376-9)
- [20] MAUREA S, LASTORIA S, CARACO C, INDOLFI P, CASLE F et al. Iodine -131 MIBG imaging to monitor chemotherapy response in advanced neuroblastoma: comparison with laboratory analysis. *J Nucl Med* 1994; 35: 1429–1435.
- [21] IZBICKI T, IZBICKA E, MAZUR J Prognostic significance of biochemical heterogeneity of catecholaminergic clones in neuroblastoma. *J Pediatr Surg* 2006; 41: 1506–15012. <http://dx.doi.org/10.1016/j.jpedsurg.2006.05.057>
- [22] STRENGER V, KERBL R, DORNBUSCH HJ, LADENSTEIN R, AMBROS PF et al. Diagnostic and prognostic impact of urinary catecholamines in neuroblastoma patients. *Pediatr Blood Cancer* 2007; 48: 504–509. <http://dx.doi.org/10.1002/psc.20888>
- [23] AYDIN GB, KUTLUK MT, YALCIN B, VARAN A, AKYUZ C et al. The prognostic significance of vanillylmandelic acid in neuroblastoma. *Pediatr Hematol Oncol* 2010; 27: 435–48. <http://dx.doi.org/10.3109/08880018.2010.489932>
- [24] OSMANAGAOGLU K, LIPPENS M, BENOIT Y, OBRIE E, SCHELSTRAETE K et al. A comparison of iodine-123 metaiodobenzylguanidine scintigraphy and single bone marrow aspiration biopsy in the diagnosis and follow-up of 26 children with neuroblastoma. *Eur J Nucl Med* 1993; 20: 1154–60. <http://dx.doi.org/10.1007/BF00171013>
- [25] IEHARA, T, HOSOI H, AKAZAWA K, MATSUMOTO Y, YAMAMOTO K, et al. MYCN gene amplification is a powerful prognostic factor even in infantile neuroblastoma detected by mass screening. *Br J Cancer* 2006; 94: 1510–1515. <http://dx.doi.org/10.1038/sj.bjc.6603149>
- [26] SPITZA R, HERO B, SKOWRON M, ERNESTUS K, BERTHOLD B et al. MYCN-status in neuroblastoma: characteristics of tumours showing amplification, gain, and non-amplification *Eur J Cancer* 2004; 40: 2753–2759. <http://dx.doi.org/10.1016/j.ejca.2004.05.002>

- [27] MORA J, GERALD WL, CHEUNG NKV Evolving significance of prognostic markers associated with new treatment strategies in neuroblastoma. *Cancer Lett* 2003; 97: 119 -124. [http://dx.doi.org/10.1016/S0304-3835\(03\)00094-6](http://dx.doi.org/10.1016/S0304-3835(03)00094-6)
- [28] ZELTZER PM, MARANGOS PJ, EVANS AE, SCHNEIDER SL Serum neuron-specific enolase in children with neuroblastoma. Relationship to stage and disease course. *Cancer* 1986; 57: 1230-4. [http://dx.doi.org/10.1002/1097-0142-\(19860315\)57:6<1230::AID-CNCR2820570628>3.0.CO;2-#](http://dx.doi.org/10.1002/1097-0142-(19860315)57:6<1230::AID-CNCR2820570628>3.0.CO;2-#)
- [29] MASSARON S, SEREGNI E, LUKSCH R, CASANOVA M, BOTTI C et al. Neuron-specific enolase evaluation in patients with neuroblastoma. *Tumor Biol* 1998; 19: 261-8. <http://dx.doi.org/10.1159/000030016>
- [30] CANGEMI G, REGGIARDO G, BARCO S, BARBAGALLO L, CONTE M et al. Prognostic value of ferritin, neuron-specific enolase, lactate dehydrogenase, and urinary and plasmatic catecholamine metabolites in children with neuroblastoma. *Onco Targets Ther* 2012; 5: 417-423.
- [31] DECAROLIS B, SCHNEIDER C, HERO B, SIMON T, VOLLAND R et al. Iodine -123 metaiodobenzylguanidine scintigraphy scoring allows prediction of outcome in patients with stage 4 neuroblastoma: results of the Cologne interscore comparison study. *J Clin Oncol* 2013; 31: 944-51. <http://dx.doi.org/10.1200/JCO.2012.45.8794>
- [32] NARANJO A, PARISI MT, SHULKIN BL, LONDON WB, MATTHAY KK et al. Comparison of ¹²³I-metaiodobenzylguanidine (MIBG) and ¹³¹I-MIBG semi-quantitative scores in predicting survival in patients with stage 4 neuroblastoma: A report from the Children's Oncology Group. *Pediatr Blood Cancer* 2011; 56: 1041-1045. <http://dx.doi.org/10.1002/psc.22991>
- [33] DUBOIS SG, KALIKA Y, LUKENS JN, BRODEUR GM, SEEGER RC et al: Metastatic sites in stage IV and IVS neuroblastoma correlate with age, tumor biology, and survival. *J Pediatr Hematol Oncol* 1999; 21: 181-189. <http://dx.doi.org/10.1097/00043426-199905000-00005>