observation in stage 4 and 4s patients aged ⩾18-months. In other words, stage 4s patients aged <18-months received less intensive treatment than stage 4 patients.

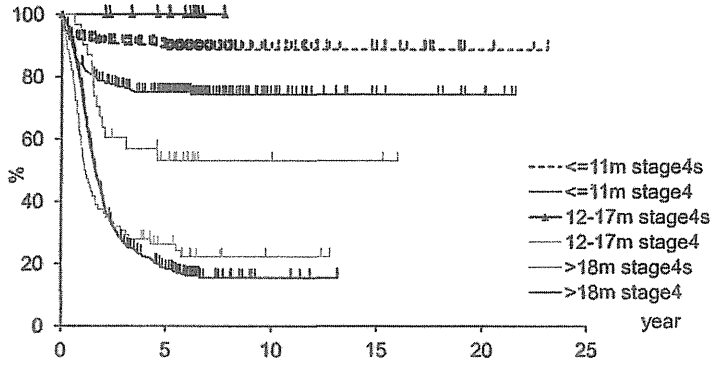
The details of prognosis of each groups were described in Table 1.The prognoses of stage 4s patients were good. Especially, the 5-year overall and event-free survival rates of the cases ⩽11 months of age, 12–17-months of age were excellent (91.2/89.4% and 100/100%, respectively) (Figs. 1 and 2). Comparing stage 4s with stage 4 in the same age, it was found that groups of stage 4s had a significantly better prognosis than the stage 4 groups (Figs. 1 and 2). For example, the *P*-values of the event-free survival rates were 0.004, 0.006 and <0.001, in patients ⩾11 months of age, 12–17-months of age and ⩾18 months of age, respectively.

4. Discussion

After Evans and DAngio reported on the uniqueness of stage 4s tumours concerning their spontaneous regression, most stage 4s tumours have been considered to be low risk tumours with an excellent prognosis.[1,2] Although there has been one report that stage 4s neuroblastoma patients do not have a poor prognosis even with *MYCN* amplification,[6] other reports have reported a poor prognosis in patients with stage 4s tumours with unfavourable prognostic factors such as *MYCN* amplification.[7–9] However, the tumours with poor prognostic factors are few in stage 4s neuroblastoma; the Children's Cancer Group Study reported that *MYCN* amplified tumour represented 0% of 80 stage 4s tumours,[10] and *MYCN* amplified cases constituted only 6% in 94 cases with stage 4s from the French Society of Pediatric Oncology.[11] In addition, it was reported that only 3.8% of all stage 4s tumours show an unfavourable histology.[10] From our results, only eight cases (3.7%) with stage 4s tumours showed *MYCN* amplification, and a few cases showed an unfavourable histology.

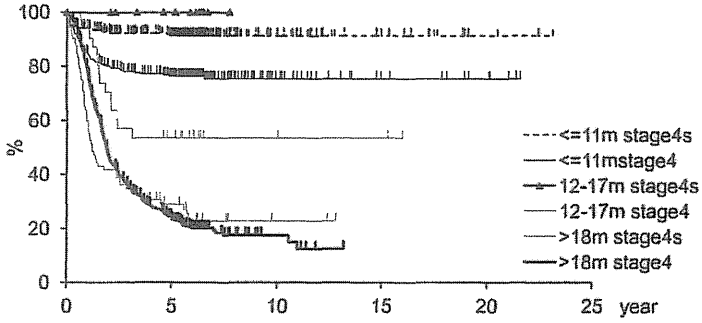
Presently, a few cases with unfavourable prognostic factors were evident in stage 4s cases involving infants both ⩽11 months of age and ⩾12 months of age. In the stage 4s patients, the serum LDH level was lower than the stage 4 patients in each group. These mean that the stage 4s neuroblastoma cases were less aggressive than the stage 4 cases at all ages were. These result that stage 4s patients who are ⩾12 months of age should have a better prognosis than stage 4 patients have, and these stage 4s cases should be different from stage 4 cases. Recently, some studies were conducted to clarify the biological difference between stage 4s and stage 4 using microarray analyses.[12,13] Although these studies did not discriminate between these stages in terms of genomic abnormalities, the possibility of a relationship between some partial chromosomal aberration, such as 17q, and clinical behaviour has been suggested.[13]

According to our research, the ratio of the stage 4s cases decreased and those of stage 4 increased with increasing age. The following two hypotheses can be suggested to the reason why the incidence of stage 4s cases changes with age. Firstly, stage 4 tumours, which are different from stage 4s tumours, developed with advancing age. The different biological characteristics of stage 4s and stage 4 tumours support this view. The number of cases detected might clinically decrease, because stage 4s tumours show spontaneous regression.[14–16] The second hypothesis assumes that stage 4s



	N	5y-EFS	
< = 11m stage 4s	273	89.4%	HRs;1.17
< = 11m stage 4	272	75.0%	95%CI;1.65-3.14, *P*=0.004
12-17m stage 4s	15	100%	HRs;3.57
12-17m stage 4	72	26.1%	95%CI;2.81-4.52, *P*=0.006
> = 18m stage 4s	31	53.1%	HRs;2.71
> = 18m stage 4	508	18.7%	95%CI;1.59-4.62, *P*<0.001

Fig. 1. Comparison of event-free survival rates of between stage 4s and stage 4 in patients <11 months old, ⩾12 to ⩽17 months, and ⩾18 months old. The event-free survival rates; The groups of stage 4s had better prognosis than the groups of stage 4 in patients <11 months old, ⩾12 to ⩽17 months, and ⩾18 months old (hazard ratios; 1.17, 3.57 and 2.71, $P = 0.001$, $P = 0.006$ and $P < 0.001$, respectively).



	N	5y-OS	
< = 11m stage 4s	273	91.2%	HRs;1.06 95%CI;1.79-4.64, P<0.001
< = 11m stage 4	272	76.3%	
12-17m stage 4s	15	100%	HRs;3.56 95%CI;2.67-4.63, P=0.007
12-17m stage 4	72	28.9%	
> = 18m stage 4s	31	53.5%	HRs;2.38 95%CI;1.40-4.06, P=0.001
> = 18m stage 4	508	24.4%	

Fig. 2. Comparison of overall survival rates of between stage 4s and stage 4 in patients <11 months old, ⩾12 to ⩽17 months, and ⩾18 months old. The overall survival rates; The groups of stage 4s had the better prognosis than the groups of stage 4 in patients <11 months old, ⩾12 to ⩽17 months, and ⩾18 months old (hazard ratios; 1.06, 3.56 and 2.38, $P < 0.001$, $P = 0.007$ and $P = 0.001$, respectively).

tumour change into stage 4 tumours, thus acquiring malignancy and thereby inducing clonal evolution.

However, it has been reported in a small number of cases that stage 4s tumours that progress to stage 4 tumours ultimately die.[17–19]

Presently, the cases with stage 4s tumours displayed a better prognosis than those cases with stage 4 tumour in infants aged ⩾12 months. Especially, the stage 4s cases aged 12–17 months had a good prognosis (100% 5 year event-free survival rate). According to the report of other countries, cases involving infants ⩾12 months of age with metastatic disease are now classified into stage 4 and receive intensive treatment.[20–22] In our study, cases ⩾12-months of age with stage 4s and 4 tumours were treated with the same induction chemotherapy consisting of cyclophosphamide and pirarubicin, cisplatin, vincristine or etoposide. The number of patients who received high-dose chemotherapy with stem cell transplantation was smaller in the stage 4s groups than in the stage 4 groups in aged 12–17-months category. As these stage 4s cases from 12–17-months of age were previously defined high risk group, they should now receive less intensive chemotherapy. It has been reported that the patients from 12–18 months of age with stage 4 non-amplified *MYCN* neuroblastoma have a better prognosis than older children.[20,21] This group may include stage 4s cases from 12 to 17 months of age. These cases are appropriate as a low risk group as well as the cases aged ⩽11 months. Therefore, the concept of stage MS of INRG of patients <18 months of age is proper.

On the other hand, the 5-year overall and event-free survival rates of stage 4s patients aged ⩾18-months were not so good (namely, 53.1% and 53.5%, respectively). This group should therefore be classified as belonging to a high risk group, and the initial intensive treatment should not be reduced.

Conflict of interest statement

None declared.

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ORIGINAL ARTICLE

Successful treatment of infants with localized neuroblastoma based on their *MYCN* status

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Abstract
Background The aim of this study was to evaluate the effectiveness of post-surgical chemotherapy for infants with localized neuroblastoma without *MYCN* amplification (MNA), and determine whether risk classification using MNA is reasonable.
Methods Four hundred and fourteen eligible patients were registered between 1998 and 2004. Resectable patients in stage 1 and 2A/2B were treated by surgical resection only. Unresectable patients in stage 3 without MNA received either 6 cycles of regimen A or 3 cycles of regimen A plus 3 cycles of regimen C2; regimen A consisted of low doses of cyclophosphamide and vincristine and regimen C consisted of cyclophosphamide, vincristine and pirarubicin before surgical resection. The resectable and unresectable patients were randomly selected to receive post-surgical chemotherapy. The patients with MNA received intensive chemotherapy regimen D2, consisting of cyclophosphamide, vincristine, pirarubicin and cisplatin, and some of them received high-dose chemotherapy with stem cell transplantation.
Results The 5-year event-free survival (5-EFS) rates of stage 1 and 2A/2B patients without MNA were 97.2 and 89.0% respectively ($p = 0.02$). A total of 31 patients in stage 3 without MNA received post-surgical chemotherapy, and 30 patients did not. The 5-EFS rates of these two groups (96.0 and 96.2%, respectively) were not significantly different ($p = 0.869$). The 5-EFS rate for localized patients with MNA ($n = 6$) was 50.0%, and that of patients without MNA was 95.0% ($p < 0.001$).
Conclusion Post-surgical chemotherapy was therefore unnecessary for localized patients without MNA. This treatment strategy using MNA is considered to be appropriate in infants.

Keywords Localized neuroblastoma · Infants · Chemotherapy · *MYCN* amplification

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Introduction

The prognosis of neuroblastoma patients improves with decreasing age, and the prognosis of infantile patients is especially good. A nationwide mass screening program for 6-month-old infants was initiated in Japan in 1985 for the early detection of neuroblastoma [1, 2], and resulted in an increased detection of early-stage infantile neuroblastoma.

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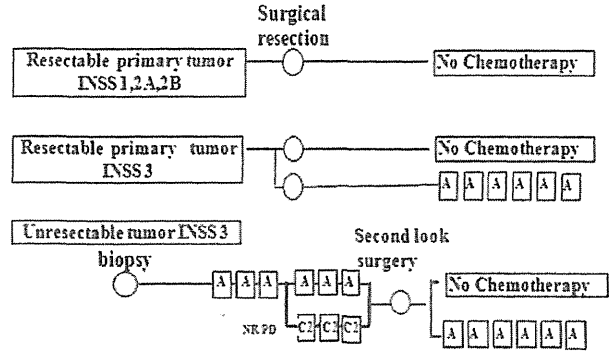
The prognoses of the patients detected in the mass screening, most of whom were infantile neuroblastoma patients, were excellent [3]. Many patients of early-stage localized neuroblastoma were found to have been unnecessarily treated with chemotherapy after surgical resection [4]. Chemotherapy was also reduced in Italy [5]. However, excessive chemotherapy was still being ordered for many localized neuroblastoma cases in infants, even though physicians in Japan in the early 1990s realized that less treatment was needed. On the other hand, *MYCN* amplification (MNA) is the most powerful prognostic factor, but there is no consensus as to whether low-stage patients with MNA should be classified a high risk. The Japanese Infantile Neuroblastoma Cooperative Study Group classifies infants with neuroblastoma using MNA and stage and assigns treatments.

The first prospective study (#9405) to examine the effectiveness of post-surgical chemotherapy for cases of stage 1 and 2 localized tumors in infants with neuroblastoma based on *MYCN* status was started in June 1994 [6]. A second study (#9805) that examined the effectiveness of post-surgical chemotherapy in localized stage 3 tumors without MNA was initiated in June 1998, and was concluded in December 2004. This paper reports on the results of this study, and discusses the effectiveness of postsurgical chemotherapy for localized neuroblastoma cases in infants.

A	Vincristine	1.5mg/m^2	day1	
	Cyclophosphamide	300mg/m^2	day8	/2W
C2	Vincristine	1.5mg/m^2	day1	
	Cyclophosphamide	600mg/m^2	day1	
	Pirarubicin	30mg/m^2	day3	/4W
D2	Vincristine	1.5mg/m^2	day1	
	Cyclophosphamide	900mg/m^2	day1	
	Pirarubicin	30mg/m^2	day3	
	Cisplatin	12mg/m^2	day1-5	/4W

Fig. 1 Chemotherapy regimens for patients with infantile neuroblastoma. Babies less than 6 months old were treated with reduced dosages: 1/3 dose for infants of less than 2 months, 1/2 dose for infants of 2–4 months, and 2/3 dose for infants ranging from 4 to 6 months of age



Abbreviation: NR, no response, PD, progressive disease

Fig. 2 Study #9805 for *MYCN* non-amplified neuroblastoma patients in stages 1, 2A, 2B and 3. Details of regimens are given in "Patients and methods". After surgical resection, stage 3 patients were randomly assigned to either receive post-surgical chemotherapy (6 cycles of regimen A) or not. *NR* no response, *PD* progressive disease

Patients and methods

Four hundred and twenty-nine patients were registered with study #9805 between June 1998 and December 2004, and 414 patients were eligible to be included in the study based on criteria. The age at diagnosis was between 0 and 14 months, and the median age was 7.35 months. Disease extension was evaluated according to the International Neuroblastoma Staging System (INSS) [7]. Histological investigation of the primary tumor was mandatory to allow diagnosis of the neuroblastoma according to the International Neuroblastoma Pathology Classification, with the central review system by the Committee of Japanese Pediatric Tumor Pathology [8]. MNA of tumor samples were detected by either a FISH (fluorescence in-situ hybridization) analysis or a Southern blot analysis, according to standard procedures [9]. Patients with 10 copies or more of the *MYCN* gene were considered to have MNA.

Treatment

Three chemotherapy regimens were used in this study, regimens A, C2, and D2 (Fig. 1). One cycle of regimen A comprised a low dose of cyclophosphamide (CPM 300 mg/m^2) on day 1 and a dose of vincristine (VCR 1.5 mg/m^2) on day 8 over a 2-week period. One cycle of regimen C2 comprised 600 mg/m^2 CPM and 1.5 mg/m^2 VCR on day 1 and 30 mg/m^2 pirarubicin (THP-ADR) on day 3 over a 4-week period. Regimen D2 comprised 900 mg/m^2 CPM and 1.5 mg/m^2 VCR on day 1 and 30 mg/m^2 THP-ADR on day 3 and cisplatin (CDDP) 12 mg/m^2 from day 1 to day 5 over a 4-week period.

The patients in stages 1, 2A and 2B without MNA were treated by surgical resection only. The resectable patients in stage 3 without MNA were randomly assigned to either receive post-surgical chemotherapy (6 cycles of regimen A) or not receive further therapy after surgical resection (Fig. 2). Unresectable patients were given either 6 cycles of regimen A or 3 cycles of regimen A followed by 3 cycles of regimen C2 to shrink the tumor, followed by

surgical resection. After surgical resection, these patients were randomly assigned to either receive post-surgical chemotherapy or not. The tumor was judged to be unresectable when damage to adjacent organs and blood vessels was expected. The final decision for resectability of tumors depended on the judgment of each institution. Patients with unresectable dumb-bell type tumors received the same chemotherapy given to patients in stage 3. Patients with MNA were classified in a high-risk group and underwent intensive treatment, with high-dose chemotherapy and stem cell transplantation. Patients with MNA were treated with 6 cycles of regimen D2, which reduced the need for high-risk induction chemotherapy [10].

Babies less than 6 months old were treated with reduced dosages: 1/3 dose for infants of less than 2 months, 1/2 dose for infants of 2–4 months, and 2/3 dose for infants of 4–6 months.

The therapeutic evaluation before surgery included a computed tomography scan or magnetic resonance imaging every three cycles. Tumors were evaluated according to the International Neuroblastoma Response Criteria (INRC) [7].

Statistical analysis

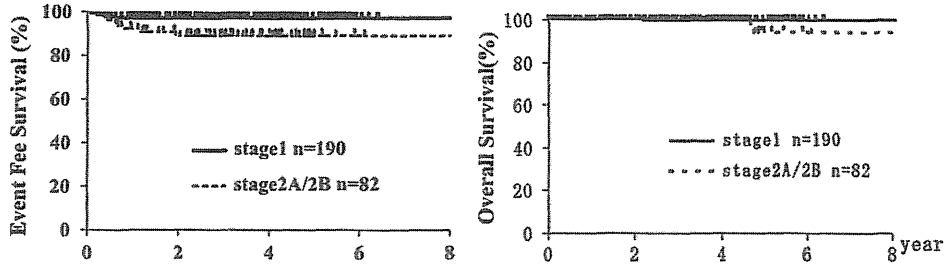
Kaplan–Meier product limit methods were used to estimate the event-free survival (EFS) and overall survival (OS) from the time of diagnosis. The exact permutation test of the log-rank statistics was used to compare the EFS and OS probabilities between subgroups of patients.

Results

There were 190 and 82 patients in stages 1 and 2 without MNA, respectively. Thirty-seven of the 82 stage 2 patients were in stage 2A and 45 were in stage 2B. Eleven unresectable patients in stage 2 were treated with 3 courses of regimen A as preoperative chemotherapy. Macroscopic residual tumors were found in 39 of all 272 patients after surgical resection. Nine patients with residual tumors received postoperative chemotherapy that was requested by the patient's family. Twenty-one patients had residual tumors at the time of prognosis.

The 5-year EFS rates of the stage 1 and stage 2 patients were 97.2 and 89.0%, respectively ($p = 0.02$). The 5-year OS rates (99.2 and 93.3%, respectively) were not significantly different ($p = 0.85$), which is excellent (Fig. 3). As to the cause of death, a stage 1 patient and a stage 2 patient died of their recurrent progressive disease after surgical resection without post-surgical chemotherapy. Surgical complications occurred in 25 infants with stage 1 and 2 neuroblastoma (9.1%). The most frequent surgical complications involved the renal urinary system ($n = 12$, 48%; Table 1).

A total of 68 patients met the criteria for stage 3 neuroblastoma without MNA in the #9805 study. Ten of 19 patients with resectable tumors received post-surgical chemotherapy and 9 patients did not receive any chemotherapy. The tumors disappeared with chemotherapy alone in 7 of the 49 patients with unresectable tumors. The remaining 42 patients underwent delayed primary surgical resection after pre-surgical chemotherapy. Twenty-one of these patients received post-surgical chemotherapy and 21 did not. A total of 31 patients received post-surgical chemotherapy and 30 did not. The 5-year EFS rates of these two groups (96.0 and 96.2%, respectively) were not significantly different ($p = 0.869$). The 5-year OS survival rates (100 and 95.8%, respectively) were not significantly different ($p = 0.306$), which is excellent (Fig. 4). Eighteen of the stage 3 patients had residual tumors at the time of prognosis. One of the stage 3 patients died of recurrent disease after surgical resection. Though the tumor sample



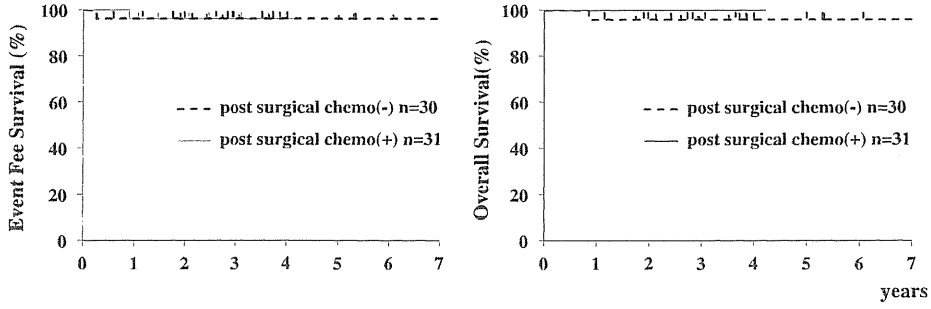
	n	5y-EFS	
Stage 1	190	97.2%	
Stage 2A/2B	82	89.0%	P=0.020
		5y-OS	
Stage 1	190	99.2%	
Stage 2A/2B	82	93.3%	P=0.850

Fig. 3 Survival rates of neuroblastoma infants in stage 1 and 2A/2B without MNA in the #9805 study. The curve was generated using the Kaplan–Meier product limit method. The 5-year event-free survival rate in stage 1 was 97.2% and 89.0% for stage 2A/2B patients ($p = 0.02$). The 5-year overall survival rate in stage 1 was 99.2% for patients and 93.3% for stage 2A/2B patients ($p = 0.85$)

Table 1 Complications of therapy

Treatment	Type of complication	Stage 3		Stage 1, 2A, 2B
		Post chem.+ 4/25 (16.0%)	Post chem.− 5/24 (20.8%)	25/272 (9.1%)
Surgery	Ileus	2	1	5
	Invagination			1
	Horner syndrome			3
	Kidney infarction			3
	Vanishing kidney			5
	Kidney resection		1	
	Hydronephrosis			1
	Renal hypertension			2
	Dysuria		1	
	Urinary tract amputation			1
	Post-operative haemorrhage			1
	Abscess			2
	Lidocaine poisoning			1
	Pulmonary effusion		1	
Chemotherapy	Neutropenia		1	
	Infectious disease	2		

Post chem.+/−: with/without post-surgical chemotherapy



Post surgical chemo	n	5y-EFS	
(-)	30	96.2%	
(+)	31	96.0%	P=0.869
		5y-OS	
(-)	30	95.8	
(+)	31	100%	P=0.306

Fig. 4 Survival rates of infants with stage 3 neuroblastoma without MNA based on post-surgical chemotherapy in the #9805 study. The curve was generated using the Kaplan–Meier product limit method. The 5-year event-free survival rate was 96.2% for patients with post-surgical chemotherapy and 96.0% for patients without chemotherapy. The 5-year overall survival rates for patients with post-surgical chemotherapy and without chemotherapy were 95.8 and 100% respectively. The 5-year event-free and overall survival rates of these two groups were not significantly different ($p = 0.869$ and $p = 0.306$, respectively)

was judged not to have MNA at diagnosis, the other sample was judged to have MNA in the later examination. It was speculated that this tumor showed heterogeneity. Four (16.0%) of the stage 3 patients who received post-surgical chemotherapy had therapy complications (Table 1). Two patients had mechanical ileus, and two had an infectious disease. Five (20.8%) of the patients who did not receive post-surgical chemotherapy had therapy complications. There were single cases of mechanical ileus, a complicated kidney resection, pulmonary effusion, dysuria and neutropenia. The frequencies of complication between patients with and without post-surgical chemotherapy were not significantly different.

There were 6 localized neuroblastoma patients with MNA (Table 2). Four patients underwent primary surgical resection at the onset, and the remaining 2 patients had stage 3 neuroblastoma. All but one of the patients received myeloablative chemotherapy with stem cell transplantation, but one infant could not receive this treatment because of his condition. The 5-year EFS rate for patients with MNA was 50.0%, and for patients without MNA was 95.0% ($p < 0.001$). The 5-year OS survival rates of patients with MNA were significantly better than without MNA (66.7 and 97.7%, respectively; $p < 0.001$) (Fig. 5).

Table 2 Characteristics of neuroblastoma patients registered with #9805 protocol

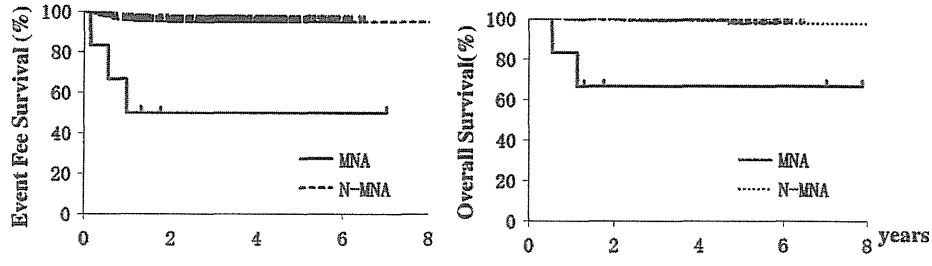
	n	MNA	%
Registered	429		
Eligible	414		
Male	238		57.5
Female	176		42.5
MS	344		83.1
Clinical	70		17.0
Median age (months)	7.35		
Stage			
1	193	3	46.7
2A/2B	38/45	1	20.0
3	70	2	16.9
4	39	3	9.4
4S	29	0	7.0
Dumb-bell	10		2.4
MNA	6		1.7
MYCN evaluable	346		

MS, mass screening; MNA, *MYCN* amplification

Discussion

The Children's Oncology Group (COG) Study treated all stage 1 and 2 patients with surgery alone regardless of the presence of MNA, although chemotherapy was recommended for patients with threatening symptoms [11, 12]. In the COG study, only 2% of stage 1 and 2 tumors exhibited MNA, and therefore no definitive conclusions were made regarding the need of the chemotherapy. However, we reported that *MYCN* is a powerful prognostic factor even in infants [13]. Recently, the International Neuroblastoma Risk Group (INRG) classification system was recommended [14]. According to the INRG classification, localized tumors with MNA were classified as high risk. Furthermore, in patients with MNA, low-stage neuroblastoma was shown to have a poor prognosis based on the INRG data [15]. This study was conducted to evaluate treatment strategies in the presence or absence of MNA in infants with localized neuroblastoma. Patients with *MYCN* amplified tumors were classified as high-risk patients. According to our results, the patients with *MYCN* amplified localized neuroblastoma actually had a poorer prognosis than patients without MNA, in spite of receiving intensive treatment. The treatment strategy using MNA is therefore appropriate in infants with localized neuroblastoma.

The Cooperative German Neuroblastoma trial NB90 treated patients in stage 1 with surgical resection and patients in stage 2 received surgical resection with 4 cycles of chemotherapy. Localized cases without symptoms and MNA did not receive chemotherapy in the new trials NB95 and NB97 [16]. Many neuroblastoma studies have therefore not tended to administer chemotherapy for neuroblastoma patients in stages 1 or 2 [17, 18]. However, the main strategy for neuroblastoma infants in stage 1 or 2 in Japan until the mid 1990s was to use chemotherapy with surgical resection. The first prospective study (#9405) was



Abbreviation: MNA, *MYCN* amplification
N-MNA, without *MYCN* amplification

	n	5y-EFS	
MNA	6	50%	
N-MNA	340	95.0%	P<0.001
		5y-OS	
MNA	6	66.7%	
N-MNA	340	97.7%	P<0.001

Fig. 5 Survival rates of infants with localized neuroblastoma based on *MYCN* status in the #9805 study. The 5-year event-free survival rates for patients with and without MNA were 50.0% and 95.0%, respectively. The 5-year overall survival rates for patients with and without MNA were 66.7% and 97.7%, respectively. The 5-year event-free and overall survival rates of patients with MNA were significantly better than those without MNA ($p < 0.001$)

therefore initiated to examine the effectiveness of post-surgical chemotherapy for patients in stage 2 without MNA. Study #9405 showed that post-surgical chemotherapy is unnecessary, and many neuroblastoma infants in the early stages were not given excessive chemotherapy. Study #9805 found the 5-year overall survival rates of stage 1 and 2 patients without MNA to be excellent.

The COG Study classified infants with stage 3 neuroblastoma without MNA as an intermediate-risk group. These patients were treated with 8–19 cycles of chemotherapy containing carboplatin, etoposide, doxorubicin and cyclophosphamide. Though the prognosis of patients under 1 year old was good in the COG study (4-year EFS, 98%), its regimen was more intensive than the regimens in the current study #9805 [19]. The French Society of Pediatric Oncology (SFOP) found low-dose chemotherapy to be sufficiently effective in infants presenting with unresectable neuroblastoma and without MNA, thus allowing for a safe surgical resection and preventing long-term late side effects. This low-dose chemotherapy included three regimens, CV (cyclophosphamide and vincristine), CE (carboplatin and etoposide) and CAdO (doxorubicin, vincristine and cyclophosphamide) [16]. Study #9805 treated unresectable patients in stage 3 with 6 cycles of regimen A, which contained a lower dose of cyclophosphamide and vincristine than was used in regimen CV. Patients received 3 cycles of regimen C2, which included pirarubicin, if the tumor was still unresectable after 3 cycles of regimen A. Only 3 patients needed to be changed to regimen C2 with pirarubicin. The low-dose chemotherapy efficiently shrank the tumor volume and prevented side effects in infants with unresectable neuroblastoma. The effectiveness of chemotherapy after surgical resection for infants with stage 3 neuroblastoma was unclear before the #9805 study. The results of study #9805 show no significant difference in survival rates of stage 3 neuroblastoma infants between patients that were and were not administered post-surgical chemotherapy.

It is clear that chemotherapy after surgical resection was unnecessary for infants with localized neuroblastoma without MNA.

Temporary neutropenia and infection were the only complications observed in these studies, and there were no regimen-related long-term late side effects. Though this is a retrospective analysis and there are several biases of institutional judgment with surgical resection, the incidence of surgical side effects in stage 3 patients was 8.8% (6/68), which was similar to that observed in another report [20]. However, some patients had long-term side effects such as kidney dysfunction and pulmonary effusion. The number of cases requiring nephrectomy among children undergoing initial resection was more than twice that in children undergoing resection after chemotherapy [21]. The International Society of Pediatric Oncology Europe Neuroblastoma Group (SIOPEN) has classified localized tumors as resectable or unresectable depending on the absence or presence of surgical risk factors. As a result, considering the surgical risk factors therefore allowed them to predict the surgical outcome [20]. The surgical risk factors are based on radiographic images at the time of diagnosis, and image-defined risk factors (IDRF) have been proposed [22]. Future studies are planned to establish surgical guidelines that consider surgical risk factors.

In Kanagawa Children's Medical Center, an observational study was tried for selected Evans stage I and II cases. From this report, in 32% of patients the tumor became undetectable and in 42% of patients the tumor was detectable without surgical resection. In 26% of patients, the tumor increased and finally underwent tumor resection [23]. Moreover, a German group reported the phenomenon of spontaneous regression of unresected neuroblastoma in infants [24]. As for some localized infantile cases, the observational strategy without surgery may be possible in expectation of spontaneous regression or maturation. It cannot be concluded that surgery is unnecessary in all patients without MNA, because some patients ultimately underwent surgical resection, since that tumor grew during observation. For the next step, we think it appropriate that we can follow up without surgery in suitable selected patients. Further investigation of biological factors during spontaneous regression is necessary.

In conclusion, this study demonstrated an effective strategy for infants with localized neuroblastoma without MNA that can result in a good clinical outcome without any side effects. The treatment strategy using *MYCN* status is therefore considered to be useful even for infants presenting with localized neuroblastoma.

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Conflict of interest All authors have no conflict of interest.

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Ijichi O); Tokyo Metropolitan Kiyose Children's Hospital, Tokyo (Hayahi A, Kamagata S); Gunma Children's Medical Center, Gunma (Tsuchida Y, Kuroiwa M); University of Tsukuba, Tsukuba (Kaneko M); Fukushima Medical University, Fukushima (Kikuta A); University of Yamagata, Yamagata (Kanazawa C); St. Marianna University of Medicine, Kawasaki (Shimada A, Okubo M); National Sapporo Hospital, Hokkaido (Hatae Y, Naito H); Hiroshima University, Hiroshima (Hiyama E, Nishimura S); Kurume University School of Medicine, Kurume (Tsuru T); Osaka University, Osaka (Fukuzawa M, Kusafuka T, Yoneda M); Nihon University School of Medicine, Tokyo (Mugishima H); National Hospital Organization, Kure Medical Center, Hiroshima (Tanaka T); Shiga University of Medicine, Shiga (Oota S).

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ORIGINAL ARTICLE

New semi-quantitative ^{123}I-MIBG estimation method compared with scoring system in follow-up of advanced neuroblastoma: utility of total MIBG retention ratio versus scoring method

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Abstract
Objective The purpose of this study is to evaluate a new semi-quantitative estimation method using ^{123}I-MIBG retention ratio to assess response to chemotherapy for advanced neuroblastoma.
Method Thirteen children with advanced neuroblastoma (International Neuroblastoma Risk Group Staging System: stage M) were examined for a total of 51 studies with ^{123}I-MIBG scintigraphy (before and during chemotherapy). We proposed a new semi-quantitative method using MIBG retention ratio (count obtained with delayed image/count obtained with early image with decay correction) to estimate MIBG accumulation. We analyzed total ^{123}I-MIBG retention ratio (TMRR: total body count obtained with delayed image/total body count obtained with early image with decay correction) and compared with a scoring method in terms of correlation with tumor markers.
Result TMRR showed significantly higher correlations with urinary catecholamine metabolites before chemotherapy (VMA: $r^2 = 0.45$, $P < 0.05$, HVA: $r^2 = 0.627$, $P < 0.01$) than MIBG score (VMA: $r^2 = 0.19$, $P = 0.082$, HVA: $r^2 = 0.25$, $P = 0.137$). There were relatively good correlations between serial change of TMRR and those of urinary catecholamine metabolites (VMA: $r^2 = 0.274$, $P < 0.001$, HVA: $r^2 = 0.448$, $P < 0.0001$) compared with serial change of MIBG score and those of tumor markers (VMA: $r^2 = 0.01$, $P = 0.537$, HVA: 0.084, $P = 0.697$) during chemotherapy for advanced neuroblastoma.
Conclusion TMRR could be a useful semi-quantitative method for estimating early response to chemotherapy of advanced neuroblastoma because of its high correlation with urine catecholamine metabolites.

Keywords Advanced neuroblastoma · ^{123}I-MIBG scintigraphy · Scoring system · Urinary vanillylmandelic acid · Urinary homovanillic acid

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Introduction

Neuroblastoma is the most common extracranial tumor in childhood. About 180–200 new cases are diagnosed each year in Japan [1]. Although some infantile neuroblastomas regress spontaneously, many advanced cases progress rapidly. In spite of aggressive chemotherapy and myeloablative chemotherapy followed by stem cell rescue, the long-term prognosis of advanced neuroblastoma remains poor, with current survival <30 % [2]. To achieve a good therapeutic outcome, it is necessary to estimate the exact tumoral extension and achieve appropriate monitoring of tumor response to treatment.

3-Iodobenzylguanidine (MIBG) is an alkylguanidine, which structurally resembles guanethidine and noradrenaline. Owing to these structural similarities, it is taken up by the specific type-1, energy- and sodium-dependent, biogenic amine uptake mechanism at the cell membrane and subsequently taken up from the cytoplasm and stored within the intracellular storage vesicles in neuroblastoma [3]. MIBG labeled with ^{123}I is widely used for neuroblastoma, and its high sensitivity and specificity have made it

an indispensable tool for examination of the staging of this disease.

The International Neuroblastoma Risk Group reported that ^{123}I-MIBG scintigraphy is mandatory and it is recommended that such analysis is performed before tumor excision [4]. Furthermore, MIBG scintigraphy is thought to be a powerful tool for estimating the response to chemotherapy. Maurea et al. [5] reported that a significant relationship was found between MIBG accumulation and urinary VMA level compared with those with serum NSE and serum ferritin in post-chemotherapy follow-up of advanced neuroblastoma.

There are some scoring methods for estimating the extent of neuroblastoma. Scoring systems are easy to use and reproducible and some previous studies have indicated that MIBG scoring systems can predict the response to chemotherapy [6–13]. While it is not doubtful that MIBG scan after induction chemotherapy may be a prognostic marker for a high likelihood of relapse, we sometimes find patients whose MIBG scores do not decrease in parallel to the rapid decreases in tumor markers after initiation of chemotherapy (Fig. 1). For these cases, another method for monitoring the response to chemotherapy is needed. Therefore, we devised a measure that reflects tumor activity itself in addition to tumor extension.

^{123}I-MIBG images are usually obtained the day after administration. In Japan, 6-hourly scans are also performed to avoid missing small metastatic lesions. Okuyama et al. [14] reported that the tumor lesions are demonstrated more clearly on an image at 30 h than at 6 h, owing to the high contrast between the RI activity of pathological accumulation in neuroblastoma and normal physiological accumulation. The difference in washout ratio of ^{123}I-MIBG from tumor and normal organs is thought to contribute to this contrast. Using this mechanism, we hypothesized that the serial change in the amount of MIBG accumulation in the entire body reflected the active tumor volume. We proposed a new semi-quantitative method for estimating the accumulations quantitatively using whole body images of two scans. In this study, the utility of this estimation method is evaluated in comparison with a scoring method and tumor markers.

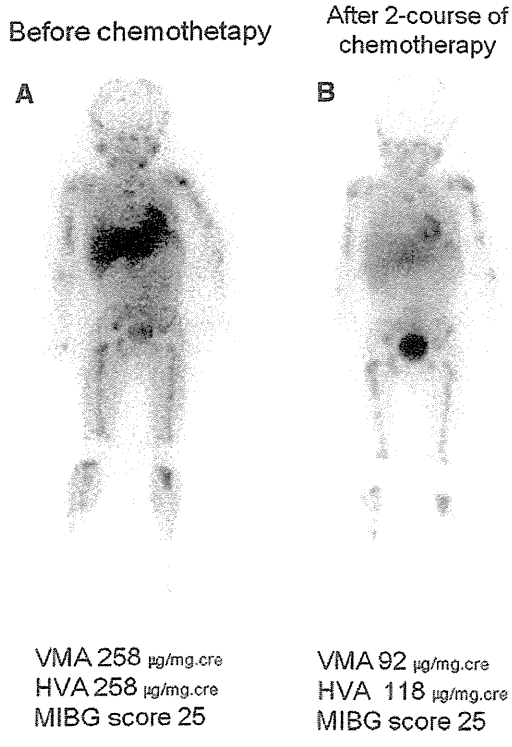

Fig. 1 Anterior planar whole body imaging of a 4-year-old boy shows ^{123}I-MIBG uptake corresponding to the right adrenal primary focus and diffuse bone/bone marrow metastases before chemotherapy (**a**) and after 2 courses of chemotherapy (**b**). As a response to chemotherapy, systemic MIBG accumulation and tumor markers decreased rapidly, but the score did not change in parallel with the decreased uptake and tumor markers

Materials and methods

Patients

Nineteen children with histologically proven advanced neuroblastoma [stage M; according to International Neuroblastoma Risk Group Staging System (INRGSS)] [4] were retrospectively included in this study. We excluded patients younger than 12 months because their prognosis is much better than older children. Five patients with metastatic disease were excluded because two of them had already undergone an MIBG study in another hospital before consulting at our hospital, and in two other cases, two scans could not be accomplished. The remaining case was MIBG-negative. Thirteen children were eventually included in this study (male/female 2/11, mean age 51.9 months, range 13–136 months at diagnosis).

Patients received intensive induction chemotherapy regimen JANB (The Japan Study Group for Advanced Neuroblastoma)91 or JANB98 for advanced neuroblastoma, with the former consisting of cisplatin, cyclophosphamide, etoposide and pirarubicin and the latter consisting of cisplatin, cyclophosphamide, vincristine and pirarubicin.

In all patients, urinary VMA, urinary HVA and serum NSE showed high values at diagnosis. For the evaluation of response to treatment, the measurements of these tumor markers and ^{123}I-MIBG scintigraphy were performed.

As a control group for the evaluation of our new method, the pretreatment MIBG results of 27 localized (INGRS: L1/L2) neuroblastoma cases (male/female 12/15,

mean age 24.2 ± 4.6 months old, range 9–30 months) were analyzed.

^{123}I-MIBG scintigraphy

^{123}I-MIBG was injected intravenously with a body weight-appropriate dose (37–90 MBq), and whole body images were obtained 5–7.5 h (early image) and 24.5–33.5 h (delayed image) after the administration. Anterior and posterior whole body scans (images 256 × 1024 pixels, scanning speed 7 cm/min) were acquired using a PRISM 2000XP or PRISM-IRIX gamma camera (Picker, Cleveland, USA) equipped with low-energy high-resolution parallel collimators. Early and delayed acquisitions were performed with the same scan speed and the same distance between the patient's body surface and the collimators. Both images in each scan were obtained in the same way using the gamma camera systems. A total of fifty-one ^{123}I-MIBG studies were performed in this study. Thirteen studies were performed at diagnosis, thirty-four studies were performed after 1–5 courses of chemotherapy and three were performed after initial surgery (mean 3.9 scans/patient). The data of ^{123}I-MIBG studies were analyzed using Odyssey-FX software.

Scoring system

For the visual estimation, in accordance with the guidelines of the Institute Curie (Paris France) [7], the whole body was divided into 9 segments to view osteomedullary involvement: (1) skull and face; (2) cervico-thoracic spine; (3) ribs, sternum and scapula; (4) lumbo-sacral spine; (5) pelvis; (6) upper arms; (7) forearms and hands; (8) upper legs; and (9) lower legs. A tenth sector that counts any soft tissue involvement was added to the score. In each area, the number or extent of abnormal accumulation was recorded as follows: 0, no site per area; 1, one site per area; 2, more than one site per area; 3, diffuse involvement (>50 % of the segment area) (Fig. 2).

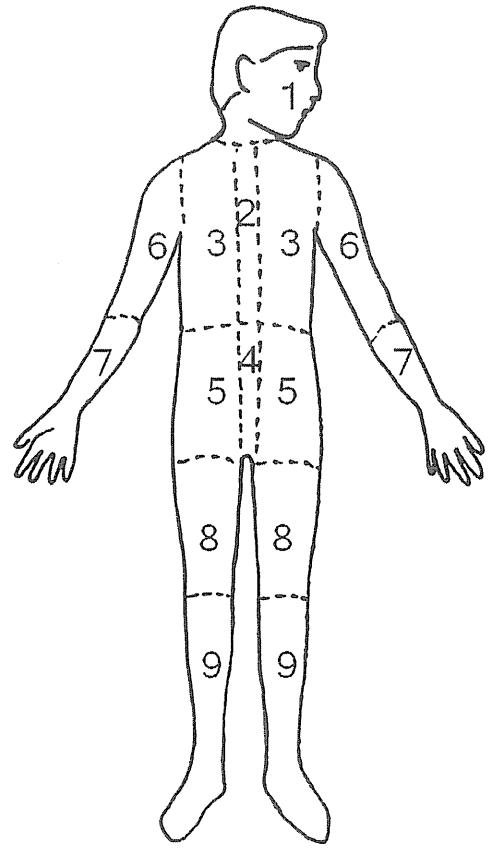

Fig. 2 ^{123}I-MIBG scoring method. This method divides the skeleton into nine segments to view osteomedullary involvement and adds a tenth sector that has any soft tissue involvement to the score

Calculation of ^{123}I-MIBG retention ratio

Using the results of anterior and posterior whole body planar images of early and delayed scans, we defined the '^{123}I-MIBG retention ratio' as follows:

$$^{123}\text{I - MIBG retention ratio} = \frac{(\text{C ant.} + \text{C post.}) \text{ of delayed scan}}{(\text{C ant.} + \text{C post.}) \text{ of early scan with decay correction}}$$

The overall score was obtained by adding the scores of the ten regions, which was defined as the 'MIBG score'. The intensity of localization was not taken into account in this study.

where cf. C ant. is the count obtained from the anterior planar image, and C post. is the count obtained from the posterior planar image).

^{123}I-MIBG retention ratio in tumors and normal organs in the control group

To compare the retention ratios in tumor and normal organs, we calculated ^{123}I-MIBG retention ratio for each region of interest (ROI) for the heart, liver, lower extremities and tumor in the control group (Fig. 3a).

Calculation of total ^{123}I-MIBG retention ratio (TMRR)

ROIs were set to encompass the entire body and RI retention in the bladder on the planar whole body image (Fig. 3b). The ^{123}I-MIBG retention ratio obtained for the whole body was defined as 'total ^{123}I-MIBG retention ratio' (TMRR):

$$\text{Total body count} = \text{whole body count (C ant.} + \text{C post.)} - \text{bladder count (C ant.} + \text{C post.)}$$

$$\text{TMRR} = \frac{\text{total body count of the delayed scan}}{\text{total body count of the early scan with decay correction}}.$$

Comparison of TMRR between stage M group and control group for initial MIBG studies

We compared the TMRR between the metastatic group (stage M) and the control group (stage L1, L2).

Correlation between TMRR, MIBG score and tumor markers in stage M group before and during treatment

We analyzed the correlations of the two estimation methods: MIBG score and TMRR, with tumor markers (urinary VMA, urinary HVA, serum NSE) for initial studies in the stage M group. To investigate whether the estimation methods reflect the response to chemotherapy, the relationships between the serial changes of the values obtained by the two methods and the change of each tumor marker were evaluated. A total of 53 MIBG studies were carried out and MIBG score and tumor markers were measured within 2 days of MIBG scan for evaluation of each chemotherapeutic course.

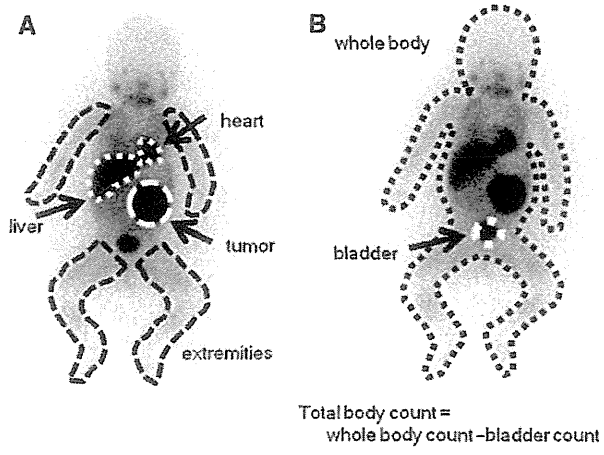

Fig. 3 **a** Regions of interest (ROIs) were set for heart, liver, extremities and tumor to calculate ^{123}I-MIBG retention ratio. **b** ROIs were set to encompass the entire body and RI retention in the bladder to calculate total ^{123}I-MIBG retention ratio

Two observers (Y.S.: radiologist, non-specialist of nuclear medicine; C.O.: radiologist, specialist of nuclear medicine) separately evaluated MIBG scores retrospectively. Interpretations were compared later in order to reach a consensus. All TMRR were calculated by one observer (Y.S.), but to evaluate the reproducibility of the new method (TMRR), two observers separately calculated TMRR of 30 randomized examinations.

Statistical analysis

The comparisons of ^{123}I-MIBG retention ratios among the organs were subjected to analysis of variance (ANOVA). Between the stage M group and the control group, differences in the mean values were assessed by Student's t test for paired data. The level of statistical significance was set at $P < 0.05$ for all tests. Data are expressed as mean ± standard deviation. Linear regression analysis was used to estimate the correlations between the two estimation methods and tumor markers.

Results

Patient characteristics

Patient data are shown in Table 1.

Reproducibility of semi-quantitative method

There was quite good inter-observer agreement ($y = 0.031 + 951X$, $r^2 = 0.793$, $P < 0.0001$) in our semi-quantitative method using 'TMRR', and it is thought that TMRR is very reproducible (Fig. 4).

^{123}I-MIBG retention ratio in tumor and normal organs in control group

^{123}I-MIBG retention ratios of heart, liver, extremities and tumor were 0.560 ± 0.139, 0.452 ± 0.128, 0.615 ± 0.086 and 1.054 ± 0.309 (mean ± SD), respectively (Fig. 5). Those of the tumor showed significantly higher values compared with those of normal organs ($P < 0.01$), while there were no statistically significant differences among those of the heart, liver and extremities.

Table 1 Patient characteristics

Characteristic	Number of patients ($n = 13$)
Gender	
Male	2
Female	11
Age (months)	
≤12	0
>12	13
Primary tumor site	
Thorax	1
Rt. adrenal	6
Lt. adrenal	6
Bone marrow involvement	
Yes	13
No	0
MYCN gene amplification	
Amplified	5
Not amplified	8

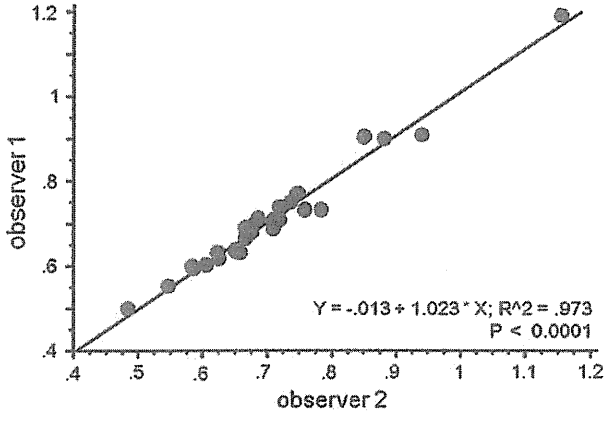

Fig. 4 There was quite good inter-observer agreement ($y = 0.031 + 951X$, $r^2 = 0.973$, $P < 0.0001$) in our semi-quantitative method using 'TMRR'

Comparison of TMRR between stage M group and control group for initial MIBG studies

TMRR values of untreated stage M patients and untreated patients in the control group (stage L1/L2) were 0.790 ± 0.182 and 0.659 ± 0.043, respectively (Fig. 6). TMRR of stage M cases showed statistically higher values ($P < 0.001$).

Correlation between tumor markers and TMRR before treatment in stage M patients

There was a positive correlation between TMRR and urinary catecholamine metabolites before chemotherapy,

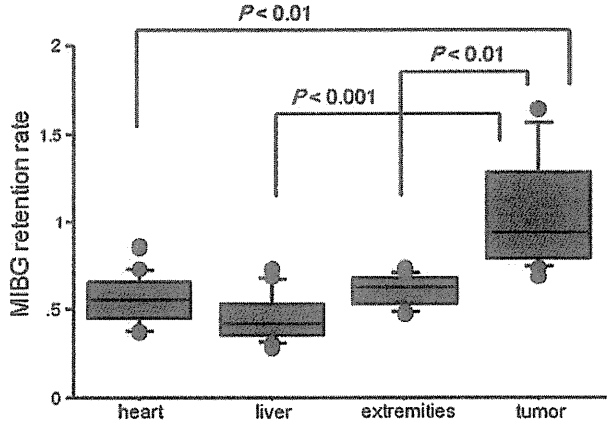

Fig. 5 MIBG retention ratios of heart, liver, extremities and tumor were 0.560 ± 0.139, 0.452 ± 0.128, 0.615 ± 0.086 and 1.054 ± 0.309 (mean ± SD), respectively. The retention ratio of tumors was significantly high compared with that of physiologic organs ($P < 0.01$)

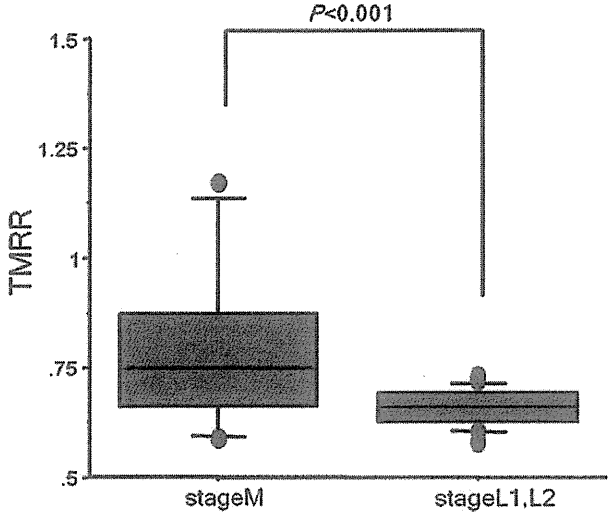

Fig. 6 TMRR of untreated stage M patients and untreated patients in the control group were 0.790 ± 0.182 and 0.659 ± 0.043, respectively. TMRR of stage M cases showed statistically higher values ($P < 0.001$)

while TMRR had a poor correlation with serum NSE (VMA: $r^2 = 0.45$, $P < 0.05$, HVA: $r^2 = 0.627$, $P < 0.01$, NSE: $r^2 = 0.032$, $P = 0.557$) (Fig. 7a–c).

Correlation between tumor markers and MIBG score before treatment in stage M patients

There was a poor correlation between MIBG score and all tumor markers before chemotherapy (VMA: $r^2 = 0.19$, $P = 0.082$, HVA: $r^2 = 0.25$, $P = 0.137$, NSE: $r^2 = 0.069$, $P = 0.387$) (Fig. 7d–f).

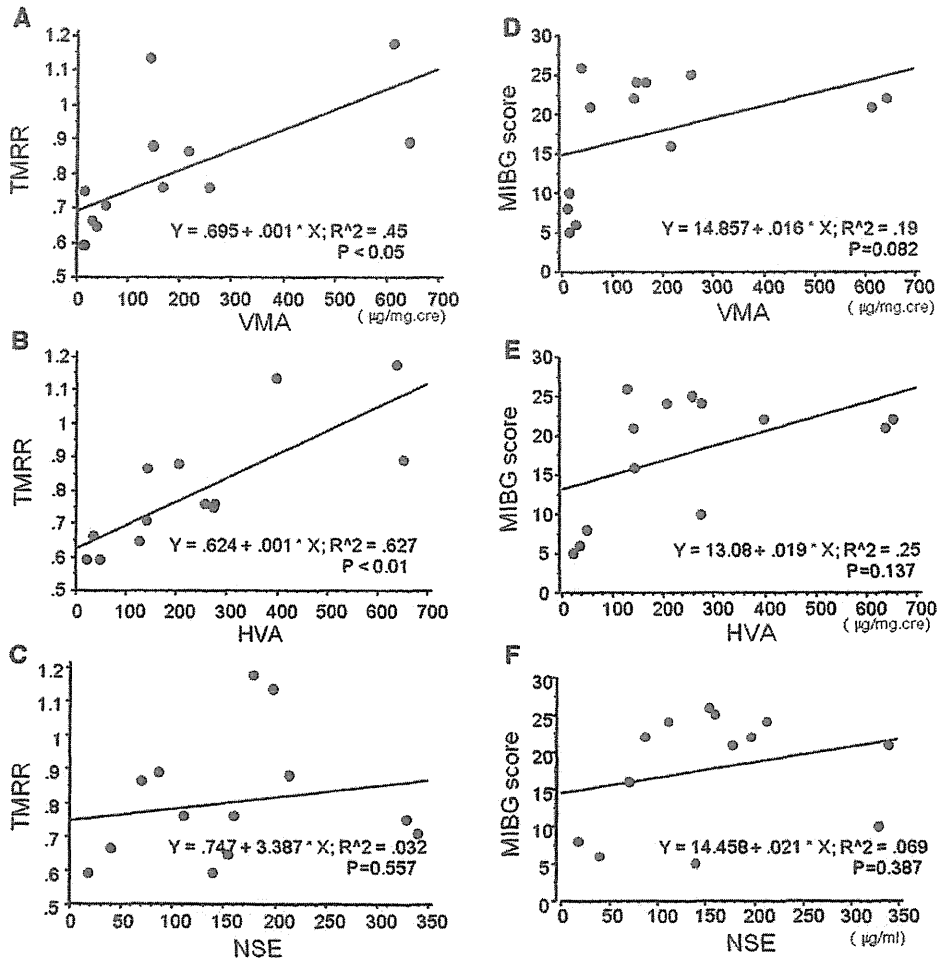

Fig. 7 **a–c** Correlations between TMRR and urinary VMA, urinary HVA and serum NSE before chemotherapy are shown (VMA: $r^2 = 0.45$, $P < 0.05$, HVA: $r^2 = 0.627$, $P < 0.01$, NSE: $r^2 = 0.032$, $P = 0.557$). **d–f** Correlations between MIBG score and urinary VMA, urinary HVA and serum NSE are shown (VMA: $r^2 = 0.19$, $P = 0.082$, HVA: $r^2 = 0.25$, $P = 0.137$, NSE: $r^2 = 0.069$, $P = 0.387$). There were tendencies for TMRR to show a higher correlation with urinary catecholamine metabolites than MIBG score before chemotherapy. Correlations between TMRR, MIBG score and serum NSE were considered to be poor in this study

Correlation between serial changes of TMRR, MIBG score and HVA/VMA during chemotherapy

We compared the result of TMRR, MIBG score and the values of urinary catecholamine after chemotherapy by using the difference with those of initial studies. The correlations between the serial change of NSE and MIBG studies were not estimated because both methods showed poor correlation to serum NSE value. The following definition was used: ΔTMRR = TMRR obtained after chemotherapy − TMRR of the initial study. ΔMIBG score, ΔVMA and ΔHVA were also defined in a similar way as ΔTMRR.

The correlations between the estimation methods (ΔTMRR and ΔMIBG score) and the urinary catecholamine metabolites (ΔVMA and ΔHVA) are shown in Fig. 8a–d. There were relatively good correlations between serial change of TMRR and those of urinary catecholamine metabolites during chemotherapy (VMA: $r^2 = 0.274$, $P < 0.001$, HVA: $r^2 = 0.448$, $P < 0.0001$). TMRR showed higher correlation with HVA than VMA, both pre- and post-chemotherapy. There were poor correlations between serial change of MIBG score and those of urinary catecholamine metabolites (VMA: $r^2 = 0.01$, $P = 0.537$, HVA: 0.084, $P = 0.697$).

Discussion

Neuroblastoma spreads hematogenously and the metastatic lesion often involves diffuse bone marrow and bones in the entire body. Nearly 70 % of patients have metastatic disease at the time of diagnosis [15].

In actively growing neuroblastoma, some markers, such as urinary catecholamine metabolites (VMA, HVA), serum NSE, ferritin and lactic dehydrogenase (LDH), usually increase. These laboratory measurements have been proposed as potentially useful for monitoring the tumor response to treatment. Although they reflect the global active status of the total tumor masses, they were not helpful in estimating the response of individual lesions to treatment.

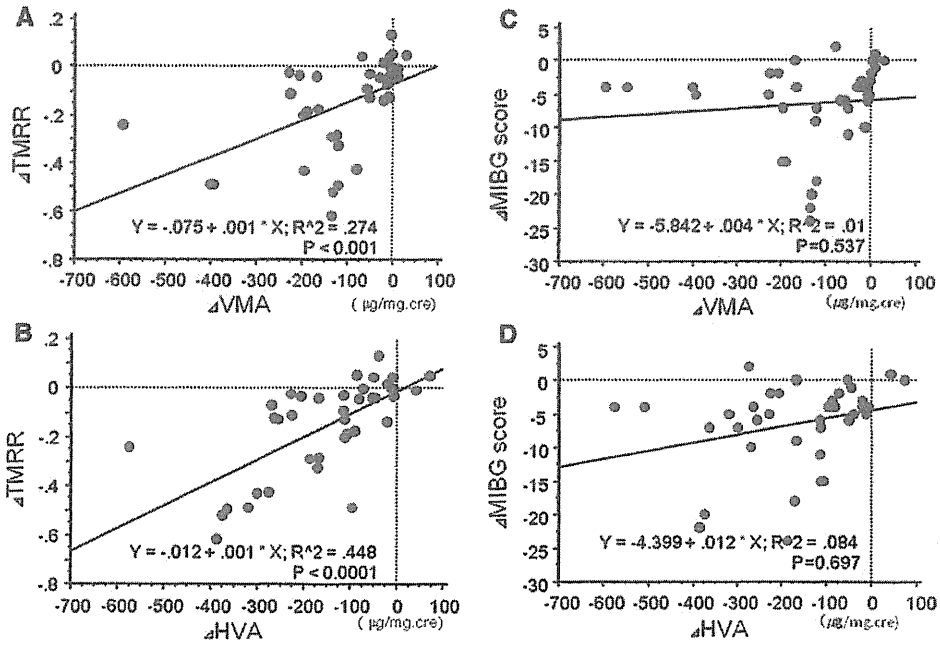

Fig. 8 **a**, **b** Correlations between serial change of TMRR (ΔTMRR) and those of urinary VMA (ΔVMA) and urinary HVA (ΔHVA) during chemotherapy (VMA: $r^2 = 0.274$, $P < 0.001$, HVA: $r^2 = 0.448$, $P < 0.0001$). **c**, **d** Correlations between serial change of MIBG score (ΔMIBG score) and those of urinary VMA and urinary HVA (VMA: $r^2 = 0.01$, $P = 0.537$, HVA: 0.084, $P = 0.697$). There were tendencies for ΔTMRR to show higher correlations with ΔVMA and ΔHVA than the ΔMIBG score

MIBG scintigraphy plays an important role in the diagnosis, staging and follow-up of neuroblastoma. It shows high sensitivity (88–93 %) and specificity (83–100 %) in detecting primary tumor and metastatic involvement (cortical bone, bone marrow and lymph nodes) [16–18]. MIBG findings can result in an increase in staging due to detection of distant disease not evident by bone marrow tests or other imaging studies such as CT or MRI. The extent of MIBG uptake might have prognostic significance.

The MIBG scoring system is a well-known method for assessing the extent of neuroblastoma and visually estimating response to chemotherapy. Scoring systems have some variations, including the division of the whole body into 7–12 segments and the assignment to each segment of scores of 0–3 or 0–6, as well as addition or lack of addition of soft tissue involvement to the score [6]. According to some reports, MIBG score at the mid-course of chemotherapy or after that showed better prognostic value than the initial MIBG score. Ady et al. [7] concluded that the MIBG score at the middle of a course of chemotherapy was strongly correlated with complete remission at the end of the induction chemotherapy. Katzenstein et al. [9] showed that patients with MIBG score >3 after induction therapy had significantly lower EFS (event-free survival) than patients with score <3.

However, from the viewpoint of monitoring chemotherapy, MIBG score does not always reflect tumor activity accurately. In advanced patients, diffuse bone marrow lesions sometimes remain after the first course of chemotherapy, even when the levels of tumor markers have decreased. In these cases, continuously high MIBG scores cannot reflect the response to treatment. Therefore, we considered how the MIBG can indicate all tumor activities. We searched for a new method for evaluating the active tumor volume and focused on the difference in the washout ratio of MIBG between the pathological accumulation in neuroblastoma and the physiological distribution in normal organs.

MIBG retention ratio (30 h uptake/6 h uptake) differed significantly between the neuroblastoma and the normal organs. As well as in the normal organs, the mechanism of MIBG uptake in neuroblastoma is considered to depend predominantly on the neuronal uptake-1 system. Once across the plasma membrane, MIBG is stored in the neurosecretory vesicles. In the pre-sympathetic neuron, the MIBG is re-excreted in the extracellular space, such as noradrenaline, when the sympathetic neuron is stimulated. However, in the tumor tissues without functional stimulation of the sympathetic nerves, MIBG would be retained longer than in normal organs. Using this difference in retention ratio, the use of 'total body MIBG retention ratio (TMRR)' was proposed. TMRR seemed to be a highly reproducible semi-quantitative method.

Stage M cases showed significantly high TMRR compared with stage L1/L2 cases. This result supports our assertion that TMRR can become a simple index of active tumor amounts. If tumors are spread throughout the body, TMRR shows a high value.

In stage M neuroblastoma patients, urinary catecholamine metabolites had better correlation with TMRR than with the MIBG score and no good correlation was found

between serum NSA and both estimation methods of MIBG. These results are consistent with the mechanism by which MIBG is stored in neurosecretory vesicles in neuroblastoma. In stage M patients, the decrease of urinary catecholamine metabolites during chemotherapy showed better correlation with the decrease of TMRR than of the MIBG score. TMRR reflects the reduced activity of the tumor as well as tumor markers and also the overall tumor burden.

On the other hand, MIBG score, which evaluates the tumor extent, did not always decrease after the initial chemotherapy. It may not adequately reflect the decrease in tumor activity in some patients whose tumor markers decreased after initial chemotherapy. These might cause the low correlation between MIBG score and tumor markers. Therefore, TMRR seems to change more closely for urine catecholamine metabolites than MIBG score during early chemotherapy and it may contribute to the evaluation of response.

Low VMA and high HVA excretions are thought to be associated with poor prognosis, whereas localized tumors usually have a VMA-to-HVA ratio of >1 [19]. In this study, TMRR showed a better correlation with HVA than with VMA, both before and during chemotherapy. As HVA is an early metabolite of the catecholamine pathway, our results may suggest that biochemically primitive neuroblastoma is more aggressive. Therefore, TMRR may be used as a good indicator of the amount of active aggressive tumor in the whole body.

Limitations of this study

This study has a bias because of its retrospective nature and the fact that a small number of cases were enrolled at a single institution.

We found that the TMRR reflects the response to chemotherapy in stage M neuroblastoma. It may be useful for advanced disease with high MIBG score, and catecholamine metabolites showed better correlation with TMRR than with MIBG score. However, TMRR could not reflect some small metastases and it failed to pick out the presence of small lesions.

Because of the variability of TMRR due to body size difference, TMRR is useful in evaluating the reactivity of chemotherapy in individual patients with diffuse metastases, not in among patients.

In this retrospective study, the scan times had variation: early images, 5–7.5 h; delayed images, 24.5–33.5 h after administration. As TMRR is found on the differences of washout between neuroblastoma tissue and normal physiological accumulation, ideally all patients should be examined at the same scan time. However, the patients could not always be prepared for the expected time, because most of them must be asleep when the scans are performed.

Since TMRR is easily calculated from the summation of anterior and posterior planar images, it cannot reflect the exact MIBG distribution in the body with complicated surface and depth. Moreover, the patient cannot always take the same posture at the two times of the scans. These findings might cause theoretical inconsistency of TMRR >1.0 in our results.

Thus, although there are some unsolved technical problems, it is expected that the serial change of TMRR can be a simple index which expresses the tumor activity of diffusely spread tumor after the treatment more precisely than MIBG scores.

The other problem is that TMRR can be influenced by diuresis and renal function; it may not always reflect the exact tumor volume when renal function is damaged by repeated chemotherapy.

Conclusion

We proposed a new semi-quantitative estimation method "TMRR" of MIBG for advanced neuroblastoma. TMRR could be useful for estimation of early response to chemotherapy of advanced neuroblastoma because of its high correlation with urine catecholamine. In addition to the extension score, TMRR is considered a helpful tool for deciding on the treatment plan such as changing of the regimen of chemotherapy and determination of additional treatment before surgery.

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REVIEW ARTICLE

Molecular and genetic bases of neuroblastoma

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Abstract Neuroblastoma, which is derived from the sympathetic nervous system, is the second most common pediatric solid malignant tumor. This pediatric tumor has a heterogeneous course, ranging from spontaneous regression to inexorable progression and death, depending on the biological features of the tumor. Identification of risk groups on the basis of clinical and molecular prognostic variables has allowed tailor-made therapy to improve outcomes and minimize the risk of deleterious consequences of therapy. In Japan, current therapeutic stratification of patients with neuroblastoma is based on risk assessment according to combinations of age, tumor stage, *MYCN* status, DNA ploidy status, and histopathology; however, unfavorable neuroblastoma is still one of the most difficult tumors to cure, with only 40 % long-term survival despite intensive multimodal therapy. Further refined therapeutic stratification based on newly identified prognostic factors will be required to improve the outcome of patients with unfavorable neuroblastoma and reduce the side effects of therapies for patients with favorable neuroblastoma. In the present review, we describe recent topics on the molecular and genetic bases of neuroblastoma; we hope this review will be helpful for understanding the mechanism of neuroblastoma tumorigenesis and aggressiveness and for developing a new therapeutic stratification and new protocols for neuroblastoma treatments.

Keywords Neuroblastoma · Molecular and genetic abnormality

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Clinical and biological features of neuroblastoma

Neuroblastoma (NB) is one of the most common malignant solid tumors occurring in infancy and childhood and accounts for 10 % of all pediatric cancers [1–3]. NB is diagnosed at a median age of about 17 months and can arise anywhere along the sympathetic nervous system, with the majority of the tumors occurring in the adrenal medulla. NB tumors in the neck or upper chest can cause Horner's syndrome (ptosis, miosis, and anhidrosis). NB tumors along the spinal column can expand through the intra-foraminal spaces and cause cord compression, resulting in paralysis. Higher-stage NBs often infiltrate adjacent organs, surround critical nerves and vessels, and are largely unresectable at the time of diagnosis, although many lower-stage NBs are encapsulated and can be surgically excised. Advanced NBs typically metastasize to regional lymph nodes and to the bone marrow via the hematopoietic system. NB cells metastatic to marrow can infiltrate cortical bone. NBs can also metastasize to the liver, most notably in patients with stage 4S tumors, which occur in about 5 % of cases. These infants have small localized primary tumors with metastases to the liver, skin, or bone marrow that almost always spontaneously regress [4].

The overall prognosis of patients with NB has markedly improved, with 5-year survival rates increasing from 52 % from 1975 through 1977 to 74 % from 1999 through 2005,