表10 TESSスコア

5-point scale

1	Impossible to do
2	Extremely difficult
3	Moderately difficult
4	A little bit difficult
5	Not at all difficult
888	Not applicable for me

Upper Extremity Questionnaire

During the past week :	
1	Putting on a pair of pants
2	Tying shoe lace
3	Putting on socks or stockings
4	Showering
5	Dressing my arms and upper body
6	Buttoning a shirt
7	Tying a tie or a bow at the neck of a blouse
8	Putting on make-up or shaving
9	Brushing your teeth
10	Brushing your hair
11	Doing light household chores
12	Gardening or yard work
13	Preparing and serving meals
14	Cutting food while eating
15	Drinking from a glass
16	Performing heavy household chores
17	Going shopping
18	Giving or receiving change (i.e. coins or bills)
19	Carrying a shopping bag or briefcase
20	Lifting a box to an overhead shelf
21	Turning a key in a lock
22	Pushing or pulling open a door
23	Writing
24	Picking up small items
25	Completing my usual duties at work
26	Working my usual number of hours
27	Participating in my usual leisure activities
28	Socializing with friends and family
29	Participating in my usual sporting activities

Lower Extremity Questionnaire

During the past week :	
1	Putting on a pair of pants
2	Putting on shoes
3	Putting on socks or stockings
4	Showering
5	Light household chores such as tidying and dusting
6	Gardening and yard work
7	Preparing and serving meals
8	Going shopping
9	Heavy household chores such as vacuuming and moving furniture
10	Getting in and out of bathtub
11	Getting out of bed
12	Rising from a chair
13	Kneeling
14	Bending to pick something up off the floor
15	Walking upstairs
16	Walking downstairs
17	Driving
18	Walking within the house
19	Walking outdoors
20	Sitting
21	Walking up or down hills or a ramp
22	Standing upright
23	Getting up from kneeling
24	Getting in and out of a car
25	Participating in sexual activities
26	Completing my usual duties at work
27	Working my usual number of hours
28	Participating in my usual leisure activities
29	Socializing with friends and family
30	Participating in my usual sporting activities

（文献21～23より）

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Original Article

Analysis of Radiotherapy in 1054 Patients with Primary Central Nervous System Lymphoma Treated from 1985 to 2009

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Abstract

Aims: Data on primary central nervous system lymphoma that had been collected through surveys for four consecutive periods between 1985 and 2009 were analysed to evaluate outcomes according to treatment.
Materials and methods: All had histologically proven disease and had received radiotherapy. No patients had AIDS. Among 1054 patients, 696 died and 358 were alive or lost to follow-up. The median follow-up period for surviving patients was 37 months.
Results: For all patients, the median survival time was 24 months; the 5 year survival rate was 25.8%. Patients treated with methotrexate-based chemotherapy and radiation had a higher 5 year survival rate (43%) than those treated with radiation alone (14%) and those treated with non-methotrexate chemotherapy plus radiation (20%), but differences in relapse-free survival were smaller among the three groups. The 5 year survival rate was 25% for patients treated with whole-brain irradiation and 29% for patients treated with partial-brain irradiation ($P = 0.80$). Patients receiving a total dose of 40–49.9 Gy had a higher 5 year survival rate (32%) than those receiving other doses (21–25%, $P = 0.0004$) and patients receiving a whole-brain dose of 30–39.9 Gy had a higher 5 year survival rate (32%) than those receiving ≥40 Gy (13–22%, $P < 0.0005$). Patients receiving methotrexate-based chemotherapy and partial-brain radiotherapy (≥30 Gy) had a 5 year survival rate of 49%.
Conclusions: The optimal total and whole-brain doses may be in the range of 40–49.9 and <40 Gy, respectively, especially in combination with chemotherapy. Patients receiving partial-brain irradiation had a prognosis similar to that of those receiving whole-brain irradiation. With methotrexate-based chemotherapy, partial-brain radiotherapy may be worth considering for non-elderly patients with a single tumour.
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Key words: Brain neoplasm; central nervous system; chemotherapy; lymphoma; neurocognitive function; radiotherapy

Introduction

Treatment strategies for primary central nervous system lymphoma (PCNSL) are gradually changing. Previously, radiotherapy played the most important role. PCNSL responds relatively quickly to radiotherapy, and the complete disappearance of enhancing tumour masses is frequently observed after radiotherapy. However, local recurrence in the irradiated volume as well as remote central nervous system (CNS) recurrence outside the treatment volume are frequently observed, and so the reported outcome of patients treated by radiation alone was relatively poor [1–3]. In addition, a proportion of PCNSL patients treated with radiotherapy develop neurocognitive dysfunction and/or show a reduced performance status [3–6]. These observations led neuro-oncologists to use systemic chemotherapy after the late 1970s.

The combination of radiation and standard chemotherapy regimens used for systemic lymphoma was attempted, but it did not yield markedly favourable results [7–11]. Subsequently, high-dose methotrexate (MTX)-containing regimens proved to be effective [12–16]. As long-term remission is often achieved with such chemotherapy, a recent trend has been to treat PCNSL with MTX-based chemotherapy first, and reserve radiotherapy for recurrence, especially in elderly patients [17,18]. However, higher

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rates of progression-free survival were noted with the use of radiation in the first-line treatment in a randomised European trial comparing chemotherapy with chemo-radiotherapy [19]. Therefore, the optimal treatment for PCNSL still remains to be determined; the role of radiotherapy should be clarified, especially in non-elderly patients, and optimal forms of radiotherapy, with regard to the treatment volume and radiation dose, should be investigated.

Until very recently, radiotherapy was used in the first-line treatment of PCNSL in Japan, either alone or in combination with chemotherapy, regardless of the patient's age. To evaluate the changes in patient, tumour and treatment characteristics in PCNSL, our group has conducted surveys of Japanese PCNSL patients treated in radiotherapy departments. Data have been collected on patients treated during the four periods of 1985–1994, 1995–1999, 2000–2004 and 2005–2009. Results of respective surveys have been published [20–24]. Through the surveys, data on a total of 1054 patients with histologically proven PCNSL have been accumulated. The purpose of this study was to evaluate the treatment outcome of these patients according to the treatment modality and radiation methods.

Materials and Methods

This study was approved by the institutional review board of Nagoya City University (approval number 506). Submission of the data was approved by institutional review boards at each institution. Informed consent for the use of data for research purposes was obtained from patients. Methods for the collection of data used for this analysis have been described in detail previously [20–24]. The surveys were carried out by the Japanese Society for Therapeutic Radiology and Oncology Lymphoma Study Group (JLSG), the Chubu Radiation Oncology Group (CROG) and the Japan Radiation Oncology Study Group. Subjects of all surveys were patients with histologically proven PCNSL who received radiotherapy. Patients who were suspected of having secondary CNS lymphoma were excluded. Those who did not complete the planned radiotherapy were included.

Data on 466 patients who started radiotherapy between 1985 and 1994 were collected from 62 institutions. For the period of 1995–1999, 142 patients were accumulated from 25 institutions with the two surveys conducted by the JLSG and CROG. For the period of 2000–2004, 131 patients were accumulated from 17 institutions by JLSG and CROG. Data on 315 patients treated between 2005 and 2009 were collected from 20 institutions. Combining the data, 1054 patients were therefore the subjects of this study. Table 1 summarises the patient and tumour characteristics and treatment details. Among the 1054 patients, 449 (42.6%) and 267 (25.3%) were ≥65 and 70 years old, respectively. Among the patients, 696 died and 358 were alive or lost to follow-up. The median follow-up period for surviving patients was 37 months.

The HIV titre was negative in all patients tested, and no other patients were considered to have AIDS-related PCNSL. The extent of surgical resection had not been ascertained in the survey for 1985–1994, but it was included in the subsequent surveys. Other items were common to all surveys. The performance status before radiotherapy scored with the World Health Organization criteria was used in this analysis. The neurocognitive status of the patients during follow-up periods was asked; all investigators judged neurocognitive function from clinical and neurological symptoms, and a standard battery of neurocognitive tests was not routinely used. Responses to induction chemotherapy and salvage treatment at recurrence were not requested. As expected in such a survey, a number of items were not answered by the investigators.

Although techniques of radiotherapy were not asked, it was confirmed in the group meetings that whole-brain irradiation was delivered using parallel opposing fields,

Table 1
Patient and tumour characteristics and treatment details

	Characteristics	Number (%)
Gender	Male/female	630(60)/424(40)
Age (years)	Median, range	62, 5–93
Performance status	0/1/2/3/4/unknown	76(8.5)/313(35)/248(28)/215(24)/38(4.3)/164
Lactate dehydrogenase	Normal/high/unknown	530(66)/276(34)/248
Phenotype	B/T/unknown	735 (95)/36(4.7)/283
Tumour number	1/≥2/unknown	580(55)/466(45)/8
Tumour size (cm) at diagnosis	Mean ± SD	3.7 ± 1.4
Surgery	Biopsy/resection/unknown	395(67)/193(33)/466
Brain irradiation field	Whole brain/partial brain	969(92)/85(8.1)
Spinal irradiation	+/−/unknown	54(5.3)/967(95)/33
Total dose (Gy)	Mean ± SD	47.8 ± 10.2
Whole-brain dose (Gy)	Mean ± SD	34.5 ± 12.1
Systemic chemotherapy	+/−/unknown	643(64)/365(36)/46
Methotrexate-based regimen	+/−	351(55)/292(45)
Intrathecal chemotherapy	+/−/unknown	98(9.9)/896(90)/60

Figures in parentheses indicate percentage of patients, excluding those with unknown data.

and partial-brain irradiation and focal boost after whole-brain irradiation were given using two to four portals from various angles or rotational fields depending on the tumour location. Partial-brain irradiation was defined as non-whole-brain irradiation; the radiation field usually included 2–4 cm margins from a tumour mass [25]. Various chemotherapy regimens were used, but, for the convenience of analysis, they were categorised as high-dose ($\geq$1 g/m^2) MTX-containing or other regimens. About two-thirds of non-MTX-containing regimens included vincristine, cyclophosphamide, doxorubicin and prednisolone [5]. Among 643 patients receiving systemic chemotherapy, 351 (54.6%) were treated with high-dose MTX-containing regimens and 292 (45.4%) received non-MTX-containing regimens. In the 351 patients receiving MTX, the MTX dose was unknown in 62. In the remaining 289 patients, the starting dose of MTX was $\geq$4 g/m^2 in 29 (10.0%), 3.5 g/m^2 in 149 (51.6%), 3 g/m^2 in 59 (20.4%) and <3 g/m^2 in 52 (18.0%).

Differences in the incidence of neurocognitive decline between paired groups were examined using the chi-squared test. Overall and relapse-free survival rates were calculated from the date of starting radiotherapy using the Kaplan–Meier method, and differences in pairs of survival curves were examined with the Log-rank test. In calculating relapse-free survival, the relapse included both intra- and extra-CNS recurrences; patients who died without tumour recurrence were censored at the time of their death. Multivariate analysis of potential prognostic factors was carried out using the Cox proportional hazards model. In doing multivariate analysis, patients were divided into two groups and all the parameters were entered as dichotomous variables. All statistical analyses were carried out using StatView Version 5 (SAS institute Inc., Cary, NC, USA) and HALWIN (Gendaisuugakusha, Kyoto, Japan).

Results

Overall and Relapse-free Survival

In all 1054 patients, the median survival time (MST) was 24 months and the 5 year survival rate was 25.8%. The absence or presence of relapse was reported in 987 patients; the median time to relapse was 14 months and the 5 year relapse-free survival rate was 22.8%. Survival rates for the respective periods have been reported previously [23,24]; briefly, the 5 year survival rate was 15.3% for the period 1985–1994, 29.5% for 1995–1999, 30.4% for 2000–2004 and 36.5% for 2005–2009. As the patients treated after 1995 and those treated with high-dose MTX-containing chemotherapy seemed to show a more favourable prognosis, these two groups of patients were separately examined in a number of the following analyses, but, as a result, general trends did not change significantly.

Outcome According to Treatment Modality

Patients were classified into three groups according to treatment modality: (1) radiotherapy alone; (2) non-MTX chemotherapy plus radiation; (3) MTX-based chemotherapy plus radiation. Figure 1A shows overall survival curves for the three groups. The MST was 14, 23 and 40.5 months for groups 1, 2 and 3, respectively, and the 5 year survival rate was 13.8, 20.4 and 42.7%, respectively (P < 0.0001). Patients treated with MTX-based chemotherapy plus radiation had markedly high survival rates. As patient selection biases existed among the three groups, patients with an age < 70 years and a performance status 0–2 and receiving radiation doses $\geq$30 Gy were analysed (defined as the favourable-prognosis group). However,

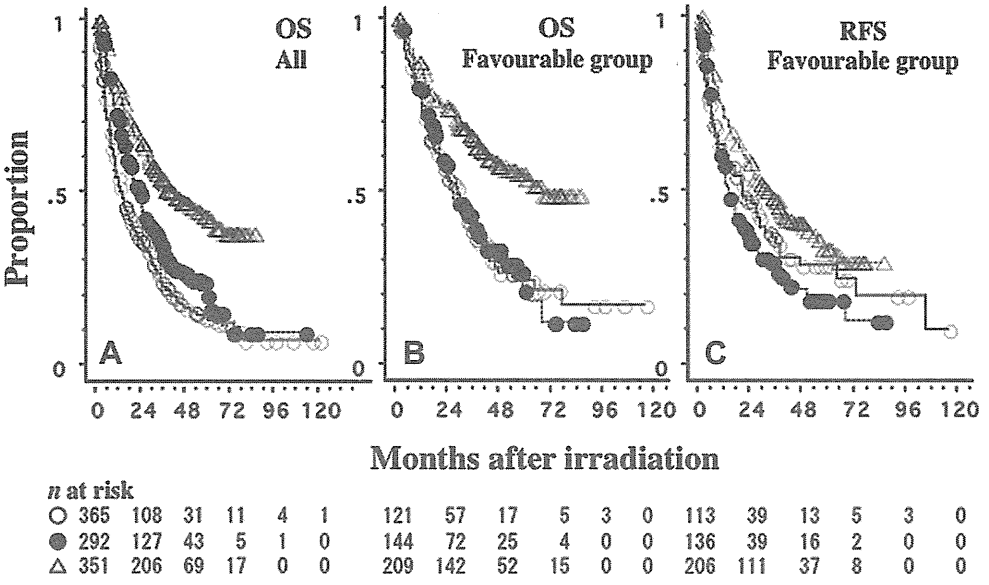

Fig 1. Outcome according to treatment modality. ○, radiation alone; ●, non-methotrexate chemotherapy plus radiation; △, methotrexate-based chemotherapy plus radiation. (A) Overall survival: ○, n = 365; ●, n = 292; △, n = 351; P < 0.0001. (B) Overall survival for favourable-prognosis patients (age < 70 years, performance status 0–2 and a total radiation dose $\geq$ 30 Gy); ○, n = 121; ●, n = 144; △, n = 209; P < 0.0001. (C) Relapse-free survival for favourable-prognosis patients; ○, n = 113; ●, n = 136; △, n = 206; P = 0.0004.

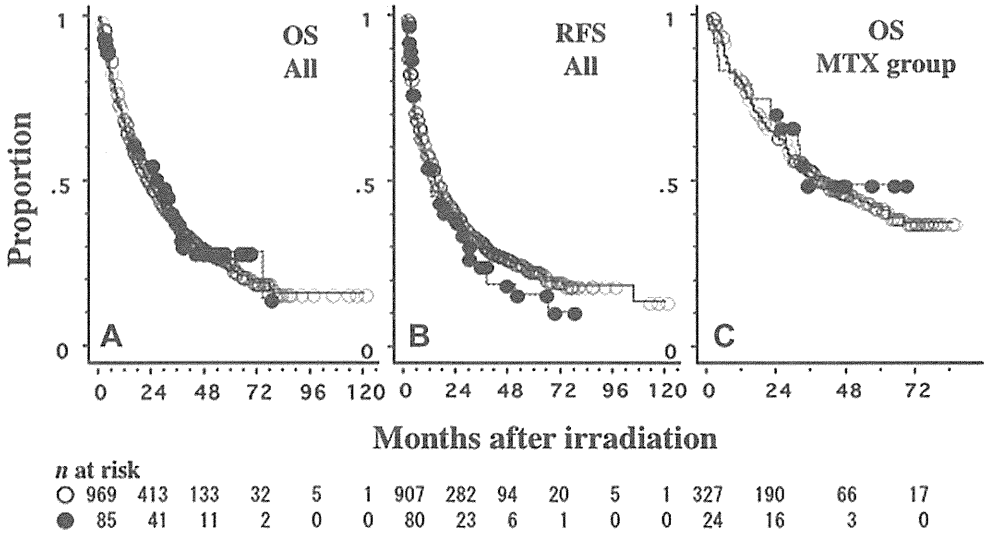

Fig 2. Outcome according to radiation treatment volume. ○, whole-brain irradiation; ●, partial-brain irradiation. (A) Overall survival for all patients (○, $n = 969$; ●, $n = 85$; $P = 0.80$); (B) relapse-free survival for all patients (○, $n = 907$; ●, $n = 80$; $P = 0.35$); (C) overall survival for patients receiving methotrexate-based chemotherapy plus radiation (○, $n = 327$; ●, $n = 24$; $P = 0.77$).

results were similar, as shown in Figure 1B. Relapse-free survival curves for the three groups in the favourable-prognosis group are shown in Figure 1C. Differences among the three groups were smaller compared with overall survival curves.

Outcome According to Radiation Treatment Volume

As an initial radiation field, 969 patients (91.9%) received whole-brain irradiation and 85 patients received partial-brain irradiation; 46% of the patients with whole-brain and 25% of those with partial-brain irradiation had multiple tumours. Figure 2 shows overall and relapse-free survival curves according to the treatment volume. The MST and 5 year survival rates were 23 months and 24.8%, respectively, for the patients treated with whole-brain radiotherapy and 25.5 months and 29%, respectively, for those treated with partial-brain radiotherapy ($P = 0.80$). No difference was found between the two groups, even when an analysis was carried out of the 588 patients treated between 1995 and 2009 ($P = 0.63$; data not shown). Relapse-free survival also did not differ between the two groups (Figure 2B). The observation was the same in patients treated with MTX-based chemotherapy plus radiation (Figure 2C), and patients treated with MTX-based chemotherapy plus partial-brain radiation had a 5 year survival rate of 49%.

When only patients with a single tumour were analysed, the overall survival did not differ between the whole-brain irradiation-treated and partial-brain irradiation-treated patients (5 year survival rate: 28.6 versus 29%, respectively; $P = 0.54$). However, relapse-free survival rates were higher in the former than in the latter (5 year relapse-free survival: 31.0 versus 14%, respectively; $P = 0.022$). When only patients with a single tumour treated with MTX-based chemotherapy were analysed, overall and relapse-free survival did not differ between the whole-brain irradiation-treated and partial-brain irradiation-treated patients (5 year survival rate: 52.6 versus 47%, respectively, $P = 0.53$; 5 year relapse-free survival: 45.0 versus 33%, respectively, $P = 0.33$).

Outcome According to Total Radiation Dose

Patients were divided into four groups according to the total radiation dose: (1) 30–39.9 Gy; (2) 40–49.9 Gy; (3) 50–53.9 Gy; (4) ≥54 Gy. Patients receiving < 30Gy were not included, as planned radiotherapy did not seem to be completed in most of these patients (most of them died soon thereafter, and they had a MST of only 1 month and a median time to progression of 2 months). The MST and 5 year survival rate were significantly more favourable in the group receiving 40–49.9 Gy than in the other groups (Figure 3A; 5 year survival rate 24, 31.6, 24.7 and 20.6% for groups 1–4, respectively; all $P < 0.05$ against group 2 receiving 40–49.9 Gy). Relapse-free survival data were similar to overall survival data (Figure 3B). Even when the analysis was limited to patients receiving high-dose MTX-based chemotherapy and radiation, the survival rate was similarly the highest in the group receiving 40–49.9 Gy (Figure 3C; 5 year survival rate 41, 51.1, 36.8 and 37% for groups 1–4, respectively).

Outcome According to Whole-brain Dose

Patients were divided into four groups according to the whole-brain dose: (1) 0–29.9 Gy; (2) 30–39.9 Gy; (3) 40–49.9 Gy; (4) ≥50 Gy. Patients receiving a total dose of <30 Gy were excluded, because they otherwise belong to group 1 and had an extremely short MST (as stated above). The MST and 5 year survival rate were significantly more favourable in the group receiving 30–39.9 Gy than in the other groups (Figure 4A; 5 year survival rate 28.0, 32.0, 21.7 and 9.4% for groups 1–4, respectively; all $P < 0.05$ against

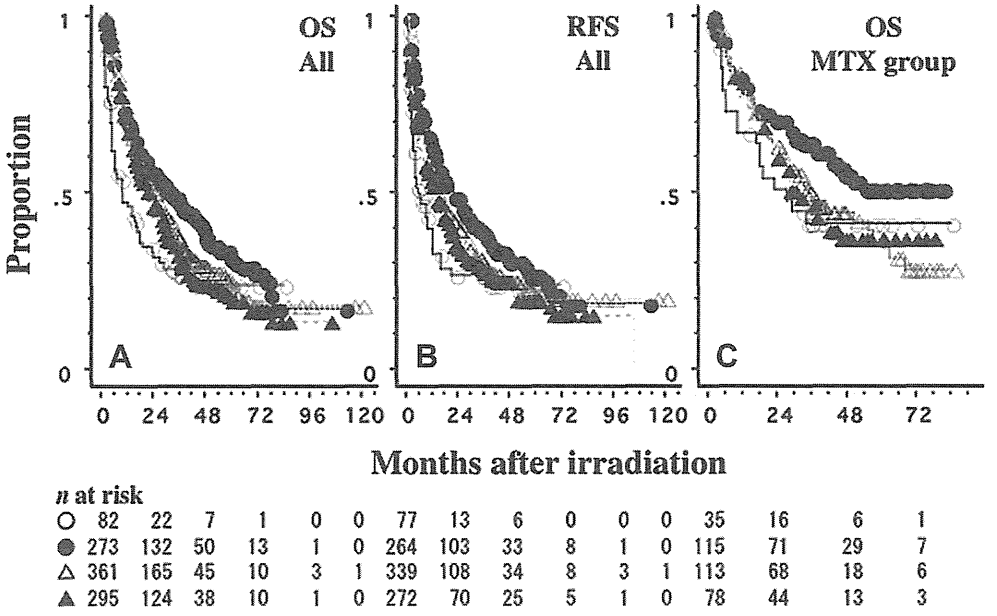

Fig 3. Outcome according to total radiation dose. ○, 30–39.9 Gy; ●, 40–49.9 Gy; △, 50–53.9 Gy; ▲, ≥54 Gy. (A) Overall survival for all patients (○, $n = 82$; ●, $n = 273$; △, $n = 361$; ▲, $n = 295$; $P = 0.0004$); (B) relapse-free survival for all patients (○, $n = 77$; ●, $n = 264$; △, $n = 339$; ▲, $n = 272$; $P = 0.0046$); (C) overall survival for patients receiving methotrexate-based chemotherapy plus radiation (○, $n = 35$; ●, $n = 115$; △, $n = 113$; ▲, $n = 78$; $P = 0.093$).

group 2 receiving 30–39.9 Gy). Relapse-free survival curves were similar (Figure 4B). However, when the analysis was limited to patients receiving MTX-based chemotherapy and radiotherapy, the survival rate was similarly higher in the groups receiving 0–29.9 Gy and those receiving 30–39.9 Gy than in the other groups (5 year survival rate 53, 45.5, 37.9 and 0% for groups 1–4, respectively).

Prognostic Factor Analysis

The 5 year survival rate according to various patient or tumour characteristics and treatment factors is summarised in Table 2. For patients ≥65 years old, the 5 year survival rate was 13.7% and the 5 year relapse-free survival rate was 20.3%. For patients ≥70 years old, these rates were 7.7% and 16.8%,

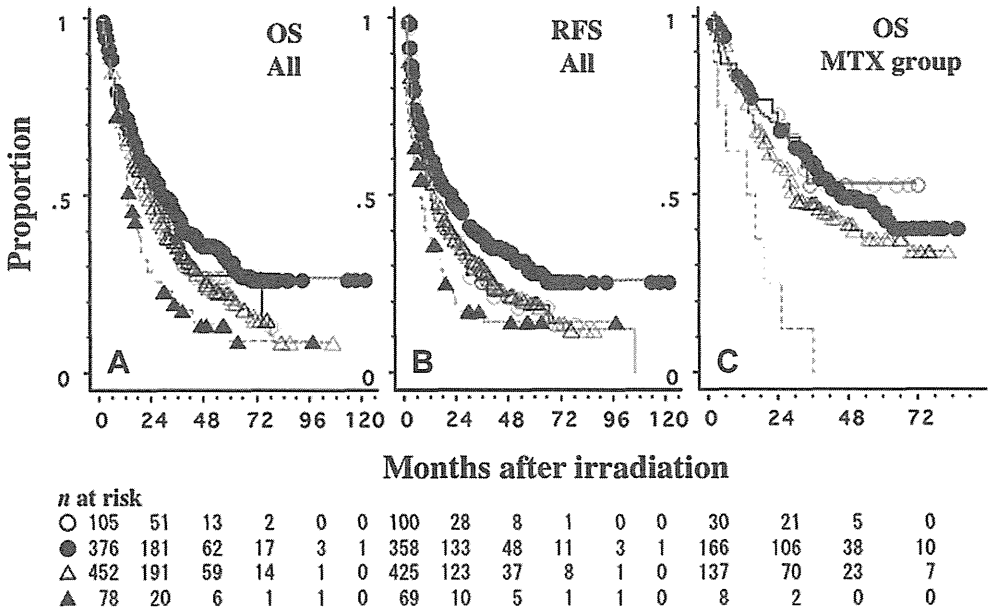

Fig 4. Outcome according to whole-brain dose. ○, 0–29.9 Gy; ●, 30–39.9 Gy; △, 40–49.9 Gy; ▲, ≥50 Gy. (A) Overall survival for all patients (○, $n = 105$; ●, $n = 376$; △, $n = 452$; ▲, $n = 78$; $P < 0.0001$); (B) relapse-free survival for all patients (○, $n = 100$; ●, $n = 358$; △, $n = 425$; ▲, $n = 69$; $P = 0.0001$); (C) overall survival for patients receiving methotrexate-based chemotherapy plus radiation (○, $n = 30$; ●, $n = 166$; △, $n = 137$; ▲, $n = 8$; $P = 0.0004$).

Table 2
Five year survival rate according to patient/tumour characteristics and treatment factors

	Characteristics	5 year survival (%)	*P*
Gender	Male/female	26.6/22.8	0.30
Age (years)	<60/60–69/≥70	37.6/19.3/7.7	<0.0001
Performance status	0/1/2/3/4	47/35.9/24.2/15.4/5.6	<0.0001
Lactate dehydrogenase	Normal/high	35.6/17.9	<0.0001
Phenotype	B/T	26.8/29	0.62
Tumour number	1/≥2	28.8/21.0	0.0029
Tumour size at diagnosis (cm)	<4/≥4	27.4/25.8	0.21
Surgery	Biopsy/resection	35.9/29	0.24
Brain irradiation field	Whole brain/partial brain	24.8/29	0.80
Spinal irradiation	+/−	23/24.0	0.97
Total dose (Gy)	30–49.9/≥50	29.6/22.9	0.22
Whole-brain dose (Gy)	0–34.9/≥35	32.9*/21.0*	0.0023
Systemic chemotherapy	+/−	32.5/13.8	<0.0001
Methotrexate-based regimen	+/−	42.7/20.4	<0.0001
Intrathecal chemotherapy	+/−	43/24.2	0.025

* Patients receiving a total dose of <30 Gy were excluded.

respectively. Multivariate analysis was carried out for the factors listed in Table 2, excluding the three factors with $P > 0.5$. For this analysis, patients receiving <30 Gy were excluded, because of the reason stated above. The influence of chemotherapy was analysed for a MTX-containing one versus other or no chemotherapy. Figure 5 shows hazard ratios for the 11 factors. Among them, younger age, better performance status, single tumour, normal lactate dehydrogenase level and use of MTX-based chemotherapy were associated with better overall survival.

Neurocognitive Function

The presence of a decline in the neurocognitive function during the course of follow-up was asked, irrespective of its reason. Answers were obtained for 379 cases and 109 (28.8%) were reported to have developed neurocognitive decline. The percentage was 26% (19/74) for patients receiving radiotherapy alone, 27.2% (70/257) for those receiving MTX-based chemotherapy plus radiation and 42% (20/48) for those receiving non-MTX chemotherapy and radiation. The proportion tended to be higher in the last group than in the former two groups ($P = 0.10$). The proportion of patients developing neurocognitive decline was 29.5% (103/349) for those receiving whole-brain irradiation and 20% (6/30) for those treated with partial-brain irradiation ($P = 0.27$). The proportion was 23.9% (39/163) for those receiving a total dose of 30–49.9 Gy and 33.0% (69/209) for those receiving ≥50 Gy ($P = 0.055$).

Discussion

This study analysed patient data collected over 25 years; during the period, marked changes occurred regarding patient characteristics and the treatment policy. Therefore, some biases need to be taken into account when evaluating the treatment outcome according to modalities and methods. Patients treated with high-dose MTX-containing chemotherapy plus radiation had the highest overall survival rates. This group consisted of many patients treated in the newest era. Patient care and second-line treatment have improved over the years, and more recent patients tend to show better overall survival. Even when patients who were expected to have a favourable prognosis were compared, differences among the treatment modalities existed, but they became smaller when relapse-free survival was compared (Figure 1C). Randomised trials of radiation versus radiation plus MTX-based chemotherapy have not been and will not be conducted in the future, so the data in the present study will be helpful to understand the issue. In addition, this study suggested that non-MTX chemotherapy might be of no merit when combined with radiotherapy.

As PCNSL is often multiple and the margin of PCNSL lesions is obscure, whole-brain irradiation is safe in terms of not missing viable tumour cells and it has been commonly used to treat PCNSL. This was especially so in the era before magnetic resonance imaging or computed tomography. However, the toxicity of whole-brain irradiation has been

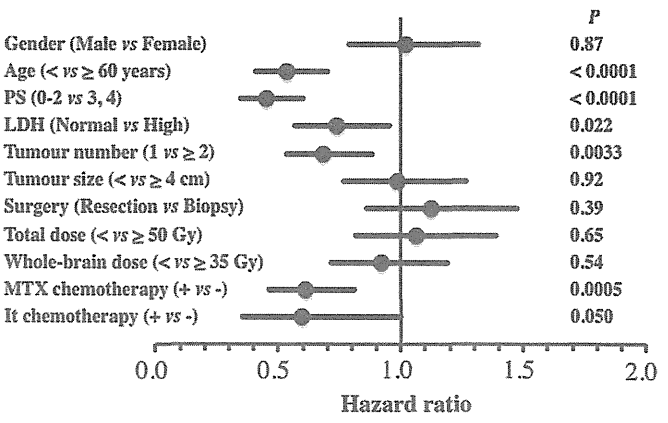

Fig 5. Hazard ratios and multivariate *P* values for the potential prognostic factors. Bars represent 95% confidence intervals. LDH, lactate dehydrogenase; It, intrathecal.

stressed [3–5]. We speculate that PCNSL grows invasively against normal brain tissue so that such normal cells, even if they retain their function at the diagnosis of PCNSL, are more vulnerable to radiation than in other tumours. Brain metastases and other tumours might invade normal tissue less aggressively, and normal cells are considered to be more resistant to the adverse effects of radiation, so that neurocognitive decline is less frequent in patients with these tumours [26,27]. Even when the whole brain is not irradiated, the critical region for neurocognitive function retention might not be outside the treatment volume, but we expect that avoiding whole-brain irradiation will contribute to retaining the overall neurological function in PCNSL patients. The results of the present study suggest that partial-brain irradiation may be considered in patients with a single tumour, especially when combined with high-dose MTX-containing chemotherapy, as high-dose MTX is expected to eradicate microscopic diseases. When employing partial-brain irradiation, the addition of wide margins up to 4 cm from a tumour mass is recommended [25].

No randomised studies have been conducted regarding the optimal total and whole-brain irradiation doses, and few non-randomised studies have addressed the issue of the optimal radiation dose. The RTOG 8315 study, with 41 patients, investigated an increase in the total dose to 60 Gy (40 Gy to the whole brain followed by a 20 Gy focal boost), but the results were not considered superior to those obtained with lower doses of radiation (e.g. 50 Gy) [1]. The results agree in part with ours, but lower doses have not been investigated. In our study, 64% of the patients had received chemotherapy, and patients receiving a total dose of 40–49.9 Gy showed the most favourable survival. Patients receiving higher doses might have had more advanced disease, leading to poorer survival, but our data, as well as the RTOG 8315 data, suggest that the effects of radiotherapy might saturate at a certain dose. In the era of MTX-based chemotherapy, we postulate that 40–45 Gy may be a reasonable radiation dose to be used in first-line treatment.

Regarding the whole-brain dose, reduction of the dose from 45 to 30.6 Gy seemed to be associated with an increased recurrence rate in a phase II study involving 57 patients [28]. On the other hand, in a phase II study of 30 patients, only 23.4 Gy was given to patients who achieved a complete response after chemotherapy, and disease control was reported to be satisfactory [29]. As the use of boost irradiation after whole-brain radiotherapy is common in Japan, our study suggested the optimal whole-brain dose to be 30–39.9 Gy. When combined with MTX-containing chemotherapy, patients receiving 0–29.9 Gy had similarly favourable prognoses. This observation supports our policy of using partial-brain irradiation combined with MTX-based chemotherapy.

The management of neurocognitive function decline after treatment is an important issue in the treatment of PCNSL. In a retrospective setting, it is difficult to evaluate neurocognitive status and, indeed, this study did not address the issue in detail, and the reported neurocognitive function decline may not necessarily be a sequela of treatment. Nevertheless, there were trends towards decreased rates of developing dementia in patients treated with partial-brain irradiation and those treated with lower total or whole-brain irradiation doses. Patients treated with MTX-based chemotherapy and radiation did not show higher rates than those with other treatments. This might be related to the use of relatively low doses of MTX; as this study included many patients treated before 2000, nearly 40% of the patients received doses <3.5 g/m^2. All these observations may also support the abovementioned treatment strategy.

Conclusions

This large retrospective analysis suggested future directions regarding radiotherapy in PCNSL treatment. If radiotherapy is integrated into primary treatment, especially for non-elderly PCNSL patients, partial-brain irradiation with less than 50 Gy doses may be worthy of consideration when the tumour occurs singly. If the long-term outcomes of currently ongoing chemotherapy-alone studies prove to be unsatisfactory, prospective randomised studies on MTX-based chemotherapy with and without partial-brain irradiation with 40 Gy should then be considered.

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ORIGINAL ARTICLE

Histological features of primary tumors after induction or high-dose chemotherapy in high-risk neuroblastoma

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Abstract
Purpose In the recent years in Japan, an increasing number of patients with neuroblastoma (NB) are being treated by the "delayed local treatment (DL)" policy, undergoing surgery after the completion of high-dose chemotherapy with hematopoietic stem cell rescue (HDC). We reviewed the histopathological findings of second-look operations, including those of patients treated with DL.
Patients From 1998 to 2013, 26 patients with high-risk NB underwent radical operation following chemotherapy. Surgery was performed after induction chemotherapy in 17 cases (standard; STD), whereas 9 cases completed induction chemotherapy and HDC before undergoing tumor resection (DL). The amount of necrosis and the degree of differentiation within the post-treatment tumor were assessed.
Results Eighty-eight percent of the tumors showed necrosis in more than 1/3 of the specimen. Two DL cases showed complete disappearance of viable tumor cells. Amount of necrosis did not affect the prognosis of the patient. Tumors with immature, poorly differentiated phenotypes showed an extremely aggressive thereafter. Though not statistically proven, ^{123}I-MIBG (metaiodobenzylguanidine) uptake may be correlated with the amount of viable cells remaining within the tumor, but not with the degree of differentiation.
Conclusions Our results support the previous reports advocating that tumors that sustain unfavorable histology after chemotherapy behave aggressively thereafter.

Keywords Neuroblastoma · Histology · Chemotherapy · Differentiation

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Introduction

Neuroblastoma (NB) is the most frequent extracranial solid tumor in childhood and originates from the sympathoadrenal lineage derived from the neural crest [1]. Despite the development of multimodal treatment including induction chemotherapy, surgical resection, high-dose chemotherapy with hematopoietic stem cell rescue (HDC), and radiotherapy, the outcome of patients with advanced NB remains poor [2, 3].

A series of clinical and biologic prognostic markers has been adopted for risk stratification of NB, and patients are generally treated following the risk-adapted therapeutic strategy. Tumor histology classification at diagnosis using the international neuroblastoma pathology classification

(INPC) [4, 5] is the mainstay of stratification, along with age [6], *MYCN* status [7], clinical stage by international neuroblastoma staging system (INSS) [8] and DNA index [9]. INPC categorizes the patients based on the pathological findings into favorable histology (FH) group and unfavorable histology (UH) group by applying the concept of age-dependent (age-appropriate) normal ranges of morphologic features. In addition to this, the classification itself regardless of age is shown to serve as a powerful tool for independently predicting the prognosis of the patient [10]. However, contrarily to the significant influence of pretreatment histological features in the treatment of NB, little has been discussed on the histology of post-treatment tumors [11–14].

In the recent decade in Japan, a unique treatment strategy following the "delayed local treatment" concept has been carried out as pilot studies in many institutions. Aiming to intensify the "dose per period" of the systemic chemotherapy regimen, the local therapy including second-look surgery and local radiotherapy is postponed until the completion of systemic chemotherapy, including HDC [15]. We have adopted this strategy as well, and found that most of the tumors remain viable at the time of surgery, leaving radiotherapy as the only postoperative treatment. This fact motivated us to investigate the post-treatment histological status within the primary tumors and its relevance to the outcome of the patient. Thus, we conducted this retrospective study including high-risk NB cases that were treated in the recent 15 years, in which patients were treated with similar induction chemotherapy protocols. The cohort included cases that underwent tumor resection at the conventional timing (after induction chemotherapy), or after the completion of HDC, to enable comparison of the different timings of surgery and the histological findings.

^{123}I-metaiodobenzylguanidine (MIBG) scintigraphies are not only valuable as a diagnostic tool [16], but also are currently implicated in the response evaluation during the treatment courses [17]. It has been reported that aggressive tumors with immature phenotype and high mitosis rate show increased ^{123}I-MIBG uptake in untreated NB tumors [18]. However, little is known about ^{123}I-MIBG uptake within primary tumors after chemotherapy and its correlation with the histological features of the tumor. In this retrospective study, we also assessed the relevance of ^{123}I-MIBG uptake prior to the radical resection of the tumor and the post-treatment histological features.

Materials and methods

Medical records of patients with high-risk NB treated with preoperative chemotherapy followed by elective surgery in two local pediatric oncology centers within the period from January 1998 to December 2013 were reviewed. Criteria of entry for this retrospective review were 1 year or older with either stage 4 disease or stage 3 with *MYCN* amplification, or under 1 year with stage 4 disease with *MYCN* amplification. A total of 26 cases met these criteria. There were eight boys and 18 girls. Median age at diagnosis was 39 months (ranging from eight to 124 months; one was under 1 year of age and the others were over one). Stage of disease (international NB staging system; INSS) was stage 3 in two patients, and stage 4 in 24 patients. The site of the original tumor was abdominal in 23 cases and mediastinal in three. All patients were initially treated with a multi-agent protocol regimen including cyclophosphamide, vincristine, pirarubicin and cisplatin. Radical resection of the primary tumor was performed in between courses of induction chemotherapy in 17 cases (standard group; STD). In 9 cases, surgery was postponed until after the completion of the entire chemotherapy regimen, including HDC (delayed local therapy group; DL). This retrospective analysis was approved by the Institutional Review Boards of the two participating institutions.

We reviewed hematoxylin–eosin-stained histologic slides of both the biopsied specimens before treatment and the post-treatment tumors for the study patients. Biopsied specimens before treatment were evaluated and classified by the international neuroblastoma pathology classification (INPC) [4, 5]. Briefly, tumors were divided into four major categories, i.e., (1) NB (schwannian stroma-poor), (2) Ganglioneuroblastoma (GNB), intermixed (schwannian stroma-rich), (3) ganglioneuroma (GN) (Schwannian stroma-dominant), and (4) GNB nodular (composite schwannian stroma-rich/stroma-dominant and stroma-poor). NB was further subdivided into undifferentiated NB, poorly differentiated NB, and differentiating NB, and GN was subdivided into GN maturing and GN mature [4].

Using the post-treatment tumor specimen, the effect of chemotherapy was evaluated in two measures: (1) amount of necrosis (including granulation tissue and fibrosis) within the entire tumor sample (2) maturation of viable neuroblasts and surrounding stroma. The amount of necrosis was quantified using the Histologic Criteria for Effectiveness of Treatment in Pediatric Solid Tumors (established by the Committee on Histological Classification of Childhood Tumors, The Japanese Society of Pathology). In the criteria, the effect of treatment is classified into four grades according to the proportion of necrosis within the tumor (Table 1). Maturation of the tumor tissue was assessed on the basis of INPC. As mentioned above, INPC is originally designed to classify tumors based on the histologic features of primary tumors in untreated patients. To adopt this system into post-treatment tumor tissue, we focused on the viable cellular components of the resected tumor and excluded the area

Table 1 Histologic criteria for effectiveness of treatment in pediatric solid tumors (established by the Committee on Histological Classification of Childhood Tumors, The Japanese Society of Pathology)

Criteria	Histological findings
Ef0	No or minimal effect
Ef1a	Mild effect: necrosis of tumor cells is observed in less than approximately one-third of the tumor volume
Ef1b	Mild to moderate effect. Necrosis is observed in one-third to two-thirds of the tumor volume
Ef2	Moderate effect. Necrosis is observed in more than two-thirds of the tumor volume
Ef3	The entire tumor is replaced with necrosis

within the tumor consisting of necrotic tissue. The morphological changes during preoperative chemotherapy were assessed by comparing INPC at the time of biopsy and at the time of radical operation.

Finally, to clarify the correlation between positive signals at the tumor site in preoperative ^{123}I-MIBG scintigraphy studies and the histological features of the subsequently removed tumor, scans obtained immediately prior to the radical operation were reviewed.

Overall survival was defined as the time from diagnosis to death. The prognostic value was evaluated by a log-rank test. Correlation of ^{123}I-MIBG scintigraphy uptake with the histological features was evaluated using Fischer's exact test.

Results

Background of patients and treatment

All patients underwent biopsy at diagnosis. Initial histological classification of the biopsied specimens before treatment according to the INPC was unfavorable histology in all cases. Pretreatment histological subtype was undifferentiated NB in three cases, poorly differentiated NB in 19, differentiating NB in one, and GNB nodular in three, according to the revised version of INPC [5]. In the three cases that were diagnosed GNB nodular, the histological diagnosis of primary tumors were GN maturing, but bone marrow aspiration revealed vivid tumor cells compatible to poorly differentiated NB, thus leading to the clinical diagnosis of GNB nodular [5].

Preoperative chemotherapy consisted of at least three cycles of induction chemotherapy, including cyclophosphamide, pirarubicin, cisplatin and vincristine, following the group study protocols in which each patient had been enrolled [19]. Five of the 26 cases received various second-line treatments before radical surgery for either stable/progressive diseases or side effects caused by initial induction chemotherapy. The median of the number of preoperative conventional chemotherapy courses given to the patients in the STD group was five (range: three to eighteen). The patients in the DL group received five or six courses of conventional induction chemotherapy followed by HDC, using either of the following preconditioning regimen: (1) melphalan, etoposide and carboplatin, (2) melphalan, etoposide and cisplatin, followed by total body irradiation, (3) mephalan and busulfan. The sources of hematopoietic stem cell were autologous peripheral blood in all cases but for two, in which allogenic bone marrow from related donors were used.

Radical surgery was aimed to obtain gross total resection in all cases, with maximal effort to preserve vital organs and major vessels. As a result, gross total resection was performed in 17 cases, subtotal resection in eight, and partial resection in two cases. In the DL group, surgery was performed during the period from day 30 to 60 post-transplantation.

Radiotherapy was used in a total of 20 cases. These included 18 cases for which local radiotherapy were used postoperatively. Three cases in the STD group received total body irradiation as a part of the preconditioning regimen for hematopoietic stem cell transplantation. None of the cases underwent preoperative radiotherapy, thus the treatment effect observed in the tumor specimens of delayed radical resection is purely a result of preoperative chemotherapy.

At the time of evaluation, eight patients were alive without disease, four were alive with disease, twelve died of the disease, and two died from treatment-related complications. The average follow-up period of the living patients was 63.5 months in the STD group, and 32.6 months in the DL group.

Histological cytotoxic effects in tumor specimens obtained in radical resection

The summarized results of the histological review of cytotoxic effects using the Histologic Criteria for Effectiveness of Treatment in Pediatric Solid Tumors are shown in Table 2. There were no tumors in the Ef0 category, suggesting that every case showed at least signs of necrosis. Tumors that were categorized into the Ef1b or Ef2 criteria accounted for 80.8 % of the total number. Fifteen cases (61.5 %) had recurrence after radical surgery. Recurrence evenly occurred in cases categorized into Ef1, Ef2a and Ef2b, suggesting that the amount of necrotic change of the primary tumor caused by preoperative chemotherapy are not correlated to a good prognosis.

Interestingly, the two cases that showed Ef3 response (complete disappearance of viable tumor cells) both belonged to the DL group. Tumors categorized either into the Ef2 or the Ef3 criteria accounted for 70 % of the DL cases,

Table 2 Cytotoxic effect measured by amount of necrosis in tumors after treatment and recurrence

Effect criteria	STD ($n = 17$)			DL ($n = 9$)		
	Total	Recurrence		Total	Recurrence	
		Local	Metastatic		Local	Metastatic
Ef0 ($n = 0$)	0	–	–	0	–	–
Ef1a ($n = 3$)	2	0	1	1	1	0
Ef1b ($n = 10$)	8	3	4	2	0	0
Ef2 ($n = 11$)	7	0	6	4	0	0
Ef3 ($n = 2$)	0	–	–	2	0	0

whereas only 44 % fell into these categories in the STD group. Although we could not draw any firm conclusion from the limited number of the cohort, the difference between the STD group and the DL group may owe to the intensified therapeutic impact of the HDC. Furthermore, the DL group appears to have less recurrence, but since the follow-up period of this subgroup is shorter than the STD group, validation of this tendency requires further follow-up.

Next, we investigated the estimated overall survival rate of patients in each effect criteria. As mentioned above, the follow-up period of the DL group was shorter than that of the STD group, and furthermore, the overall survival rate of the STD group and the DL group was not significantly different. Thus, we combined the STD group and the DL group together in the survival analyses. The overall survival of the patients also did not correlate with the histologic cytotoxic change within the primary tumors after chemotherapy. As shown in Fig. 1, the effect criteria had no relevance to the probability of survival. The two cases that showed Ef3 effects were both *MYCN*-amplified cases. Both cases are alive without disease after 74 and 37 months from diagnosis, respectively.

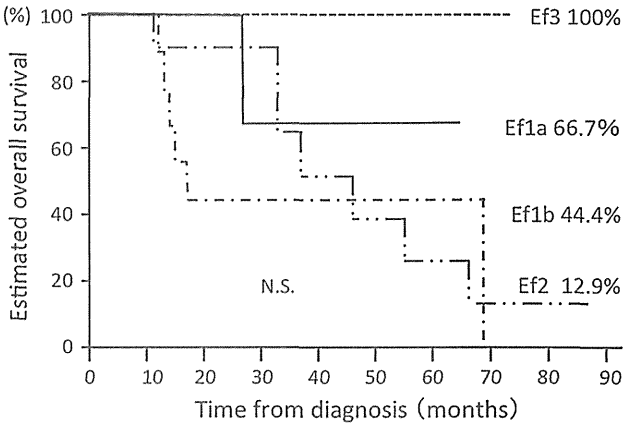

Fig. 1 Effect criteria and overall survival of patients

Histological maturation in tumor specimens obtained in delayed radical resection

The degree of maturation/differentiation of the tumor at diagnosis and at the time of radical resection was assessed based on the INPC criteria (Table 3). As expected, tumors that originally had features of differentiation at diagnostic biopsy tended to shift towards a further maturated phenotype at the time of second-look surgery, featured by enriched Schwannian stroma and maturing ganglion-like cells. On the other hand, those that originally had immature features (i.e., undifferentiated NB and poorly differentiated NB) responded to chemotherapy variously. Among the 22 tumors in these criteria, 18 tumors shifted up to either of differentiated NB, ganglioneuroblastoma (GNB) intermixed, or GN maturing. However, there were four cases that remained in the undifferentiated or poorly differentiated NB subgroup, indicating that there were no or minimum effect of differentiation/maturation within the primary tumor despite the use of preoperative chemotherapy. Table 4 shows the summary of posttreatment INPC category and the pattern of recurrence. Out of the four cases that remained in the undifferentiated or poorly differentiated NB subgroup, three were in the STD group, which all relapsed shortly after the surgery during the postoperative treatment courses (3, 5 and 7 months from surgery to relapse). Of these three patients, one underwent intraoperative radiotherapy and was subsequently treated with postoperative conventional chemotherapy, when the tumor relapsed. The other two were scheduled to receive local radiotherapy after HDC but never had the chance to do so, since local recurrence occurred shortly after HDC. The case in the DL group did not suffer recurrence but was continuously being treated for stable disease at the time of data collection. Relapse also occurred in cases that showed moderate to high degree of differentiation (i.e., differentiating NB, GNB intermixed and GN maturing categories). Twelve out of 20 cases in these categories had tumor recurrence. It is worth noting that local recurrence as the first postsurgical event was rare in this group of patients, with only one in the DL group. The first site of relapse was bone marrow or distant lymph nodes in the remaining 11 cases, which was in contrast to the relapse pattern in the poorly differentiated tumors, in which recurrence tended to occur locoregionally.

Table 3 Pre- and post-treatment tumor phenotype based on INPC criteria

Pretreatment histology of primary tumor from biopsy	Post-treatment histology of resected specimen					
	Undifferentiated NB ($n = 2$)	Poorly differentiated NB ($n = 2$)	Differentiating NB ($n = 7$)	GNB intermixed ($n = 10$)	GN maturing ($n = 3$)	No viable cell ($n = 2$)
Undifferentiated NB ($n = 3$)	1	0	0	1	1	0
Poorly differentiated NB ($n = 19$)	1	2	7	6	1	2
Differentiating NB ($n = 1$)	0	0	0	1	0	0
GN maturing ($n = 3$)	0	0	0	2	1	0

Table 4 Tumor phenotype based on INPC criteria in tumors after treatment and recurrence

Post-treatment INPC category	STD ($n = 17$)			DL ($n = 9$)		
	Subtotal	Recurrence		Subtotal	Recurrence	
		Local	Metastatic		Local	Metastatic
Undifferentiated NB ($n = 2$)	2	2	0	0	–	–
Poorly differentiated NB ($n = 2$)	1	1	0	1	0	0
Differentiating NB ($n = 7$)	5	0	4	2	1	0
GNB intermixed ($n = 10$)	8	0	6	2	0	0
GN maturing ($n = 3$)	1	0	1	2	0	0
No viable tumor cell ($n = 2$)	0	–	–	2	0	0

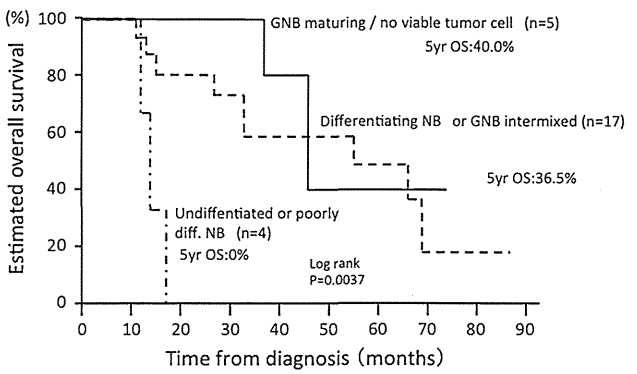

Fig. 2 Post-treatment INPC category and overall survival of patients

Overall survival of the patients in each category is shown in Fig. 2. Due to the shortage of the number of cases, each category was combined into three groups; i.e., undifferentiated/poorly differentiated NB group, differentiating NB/GNB intermixed group, and GN maturing and no viable cell group. The estimated 5-year overall survival rate of the undifferentiated/poorly differentiated NB group was 0 %, as compared to 36.5 and 40 % in the differentiating NB/GNB intermixed group and GN maturing and no viable cell group, respectively. The survival rate of the undifferentiated/poorly differentiated NB group was significantly lower than the other two groups (log-rank test; $P = 0.0037$).

Histology and ^{123}I-MIBG uptake

To clarify the correlation between preoperative imaging studies and histology of the resected tumor, we evaluated the clinical response of the local and metastatic tumors by retrospectively assessing the ^{123}I-MIBG (metaiodobenzylguanidine) scintigraphy findings. Five early cases in which ^{123}I-MIBG had not been applied routinely were excluded, and 21 cases were subjected for assessment (Table 5). Although not statistical, the cytotoxic effect and the ^{123}I-MIBG-positive rates appeared to be in an adverse relationship. Tumors that showed Ef1a or Ef1b effect tended to be ^{123}I-MIBG positive as compared to Ef2 and Ef3. On the other hand, the degree of differentiation had no apparent correlation to preoperative ^{123}I-MIBG-positive rates. The data suggests that the ^{123}I-MIBG uptake is rather dependent on the amount of viable NB cells within the tumor, regardless of the differentiation status of the NB component.

Discussion

Histological evaluation of the primary tumor in untreated advanced stage NB is mandatory for risk stratification in the current standard treatment strategies. Besides being a well-established and universally approved pathological classification, INPC also serves as a powerful predictor of

Table 5 Result of preoperative ^{123}I-MIBG scintigraphy and histological findings in tumors after treatment ($n = 21$)

	^{123}I-MIBG uptake	
	Positive	Negative
Effect criteria		
Ef1a	2	0
Ef1b	6	2
Ef2	4	5
Ef3	0	2
Resected tumor INPC		
Undifferentiated NB	1	1
Poorly diff. NB	2	0
Differentiated NB	4	1
GNB intermixed	4	4
GN maturing	1	1
No viable tumor cell	0	2

prognosis of the patients [10]. In contrast, there are only several reports in the literature focusing on the clinical value of tumor histology after the use of chemotherapy [11–14].

In the current study, we aimed to clarify the clinical impact of the histological characteristics of tumors resected at second-look surgeries. The prominent feature of our cohort is that it includes patients treated with the "delayed local treatment" policy, a multimodal treatment strategy in which the radical resection of the tumor is withheld until after the completion of all courses of chemotherapy including HDC. Local radiotherapy follows the surgery, but as a general rule, no postoperative chemotherapy courses are given. The strategy aims to avoid the prolongation of the chemotherapy intervals that could possibly be caused by performing surgery and/or radiotherapy in between the chemotherapy courses, and to consequently intensify the "dose per period" of the systemic treatment [15]. An increasing number of institutions in Japan, including ours, have adopted this strategy during the recent decade, and a nationwide multicenter group JNBSG is currently conducting a clinical study to test the feasibility and the effect of this treatment strategy. However, the strategy has raised two novel questions that had not been discussed in the past: (1) is a tumor left behind after HDC still viable? (2) if so, how would it behave afterwards? To answer these questions, we analyzed the histological features in a cohort that included patients treated with the conventional (STD) and the novel (DL) approach to compare the treatment outcomes between the two groups.

Tumor necrosis was seen in a various degree in our study, but the amount of necrosis did not correlate to the outcome of the patient. There have been reports demonstrating that increased tumor necrosis in response to preoperative chemotherapy correlates to the favorable prognosis of the patient in childhood solid tumors, such as Ewing sarcoma [20, 21], Wilms tumor [22] and osteosarcoma [23]. In neuroblastoma, the amount of necrosis in the tumor after treatment has been reported to be indicative of a better prognosis by some investigators [11, 12, 14], while Bomken et al. [13] stated that patients with tumors showing <90 % necrosis did better than those with >90 % necrosis in the post-treatment tumor. The authors indicated the possible contribution of *MYCN* amplification, which was in close correlation with post-treatment necrosis [13]. George et al. [14] commented in their recent publication that *MYCN* amplification was positively correlated with >10 % tumor necrosis at resection in their study, but these patients had an improved prognosis as compared to those with <10 % tumor necrosis. Interestingly, in our study there were eleven out of 26 cases with *MYCN* amplification, and the only two tumors that showed Ef3 effect (complete eradication of viable neuroblasts) were *MYCN* amplified. Five *MYCN*-amplified tumors showed Ef2 effect. Thus, it seems apparent that *MYCN*-amplified tumors have a trend to show more necrotic change within the post-treatment tumors, which is in agreement with the previous studies [13, 14]. Whether the amount of necrosis in the resected tumor is prognostic or not remains controversial, owing to a number of factors, including the difference in treatment intensity, timing of operation, and the cutoff of evaluation of necrotic tissue. The true value of amount of necrosis remains to be clarified by analyzing a larger cohort in a prospective manner.

Maturation of neuroblastomas into ganglioneuromatous tumors in response to chemotherapy is a well-known phenomenon. We evaluated the maturation status of post-treatment tumors adopting the INPC criteria. As a result of chemotherapy, there was an upshift toward a more differentiated phenotype in 19 of the 26 tumors. There were only four cases in which the post-chemotherapeutic phenotype was undifferentiated NB or poorly differentiated NB. Three of the four cases had an extremely poor clinical course after the surgery despite the continuation of standard postoperative multimodal therapy. This suggests that, rather than the amount of necrotic tissue within the tumor, the differentiation status of the remaining clusters of neuroblasts within the tumor tissue was more valuable as a prognostic factor in our cohort. The current study contrasts with findings in the report by George and colleagues, in which the degree of differentiation within the resected tumor (undifferentiated/poorly differentiated NB versus differentiating NB) did not have significant impact on the survival of the patients [14]. It is notable though, that in their study, 15 out of 40 cases remained in the undifferentiated/poorly differentiated NB categories after induction chemotherapy. The discrepancy between our study and theirs may have

been caused by the different periods that the patients were treated, or the different induction protocols, or the number of courses used before the surgery. Despite the negative results regarding tumor differentiation, George and colleagues found that the mitosis-karyorrhexis index (MKI) is strongly correlated to the prognosis of the patients [14]. The authors' finding, along with ours, suggests that the phenotype of the remaining tumor cell cluster after induction chemotherapy is critical for predicting the further clinical course of the patients, and additional or substantial treatment might be necessary for those that have aggressive phenotypes remaining, since these cells are likely to represent chemoresistance.

Tumors in the DL group had a trend to have a larger amount of necrosis as compared to the STD group. The intensity of chemotherapy is reported to be proportional to the amount of necrosis from previous studies [11, 12]. Our result suggests that the megadose treatment further added cytotoxic effects against the remaining tumor. However, in seven out of nine cases in the DL group, viable tumor cells were still evident. This indicates that local treatment including surgery and radiotherapy is mandatory in most cases despite the intensification of systemic chemotherapy. There is a concern that the presence of viable tumor cells at the end of chemotherapy would be a high risk of recurrence. However, the recurrence rate as well as the overall survival was not inferior in the DL group as compared to the STD group in the current study. This is in agreement with the previous report by Hashii and colleagues reporting a favorable outcome of patients treated with the "delayed local treatment" policy [15]. At this point, we do not have any firm data to know whether presence of viable tumor cells left after HDC should be a target of further treatment, but from the results of the current study, we would suggest that at least those with undifferentiated/poorly differentiated NB phenotypes after HDC may be at a high risk of recurrence and should undergo further treatment. The results of a large-scale study are warranted to clarify the clinical impact of the features of tumors resected after HDC.

Finally, we investigated the relation between preoperative ^{123}I-MIBG uptake and histological findings. Our results revealed a discrepancy in between the ^{123}I-MIBG uptake and the histological maturation of the tumor (Table 5). The uptake was evenly observed in roughly half of tumors regardless of the degree of differentiation. On the other hand, ^{123}I-MIBG uptake appeared to be correlated with the cytotoxic effect evaluated by the amount of necrosis within the tumor, though this trend was not statistically supported. It is likely that in post-treated tumors, the gross uptake decreases significantly compared to pretreated tumors, owing to the shrinkage of the tumor and the increased necrosis within the tumor. In this situation, we consider that the visible uptake would be significantly affected by the volume of the tumor and the number of viable tumor cells remaining within the tumor. Caution is therefore needed when evaluating the tumor with ^{123}I-MIBG scintigraphies after chemotherapy, since negative uptake may not guarantee the disappearance of tumor cells with unfavorable phenotypes, and the persistence of ^{123}I-MIBG-uptaking tumors not necessarily indicates the presence of aggressive tumor cells.

The weakness of the current study is that, as a nature of a retrospective institutional review, the details of treatment vary and the statistical power is low. Our findings require verification by precisely analyzing the histology of the post-treatment tumor specimens of high-risk neuroblastoma treated in a multicenter prospective clinical trial.

Conflict of interest The authors declare that they have no conflict of interest.

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Coexistence of neuroblastoma detected on staging of Langerhans cell histiocytosis

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Abstract Langerhans cell histiocytosis (LCH) is a rare proliferative disease accompanied by the accumulation of pathological Langerhans cells, which often spreads into multi-site and multi-organ systems. We here describe a girl with a history of Kawasaki disease and cervical lymphadenopathy who presented with occipital LCH. Adrenal tumor was detected on staging evaluation of LCH and was diagnosed as neuroblastoma on resection using laparoscopic surgery. Neither tumor relapsed following chemotherapy for LCH and resection of neuroblastoma. Although LCH often spreads into multi-organ lesions, invasive biopsy may be needed for tumors with atypical localization for LCH in consideration of the synchronous occurrence of malignancies.

Key words Kawasaki disease, Langerhans cell histiocytosis, laparoscopy, lymphadenopathy, neuroblastoma.

Langerhans cell histiocytosis (LCH) is a rare proliferative disease accompanied by the accumulation of pathological Langerhans cells. Its clinical manifestations have been recognized as existing along a spectrum that may involve a single site, multiple sites in a single organ system, or multiple organ systems. Stratifying patients based on their position on the spectrum has proven useful in determining prognosis and planning suitable therapy.[1]

Neuroblastoma (NB) is the most common extracranial solid tumor that occurs in childhood. NB is a disease of the sympathicoadrenal lineage of the neural crest; therefore, it can develop anywhere in the sympathetic nervous system, with a predilection for the adrenal glands.[2]

We here present the case of a girl with co-occurrence of stage 1 NB detected on staging evaluation of LCH. Although LCH may involve a multi-organ system, we need to consider the possibility of the coexistence of malignancies in the staging of LCH.

Case report

A previously healthy girl was admitted to hospital for Kawasaki disease (KD) at 18 months old. She was successfully treated with i.v. high-dose immunoglobulin with oral aspirin and recovered without coronary complications. Abdominal ultrasonography was not performed at that time. At 2 years 4 months old, she presented with bilateral lymphadenopathy, which resolved without therapy.

At 3 years old, she entered hospital for evaluation of an occipital mass, which had presented 3 months before. Skull X-ray indicated a 2 cm punched-out lesion. Cranial magnetic resonance imaging showed an intracranial-extending soft-tissue mass adjacent to the sagittal sinus (Fig. 1a), without invasion into the central nervous system. Excisional biopsy specimen confirmed the diagnosis of LCH on histological analysis including S100 and CD1a stains. Computed tomography (CT) to define organ involvement showed a right adrenal tumor (2.5 cm × 3.0 cm). The tumor presented isointensity with the liver on T1-weighted imaging, high intensity on T2-weighted imaging, and Gd enhancement (Fig. 1b). The tumor had high ^{67}Ga scintigraphy activity and positive ^{99m}Tc HMDP uptake on bone scintigraphy. Urinary homovanillic acid (HVA) was 3.27 mg/g Cr (normal, 3.5–11.9 mg/g Cr); urinary vanillylmandelic acid (VMA), 2.63 mg/g Cr (normal, 4.1–19.3 mg/g Cr); and serum neuron-specific enolase, 15.73 ng/mL (normal, 0–10 ng/mL).

Because the localization of LCH uncharacteristically involved the adrenal glands, the right adrenal tumor was resected using laparoscopic surgery for additional diagnosis. The histological diagnosis was NB, differentiating, according to International Neuroblastoma Pathology Classification. The tumor sample

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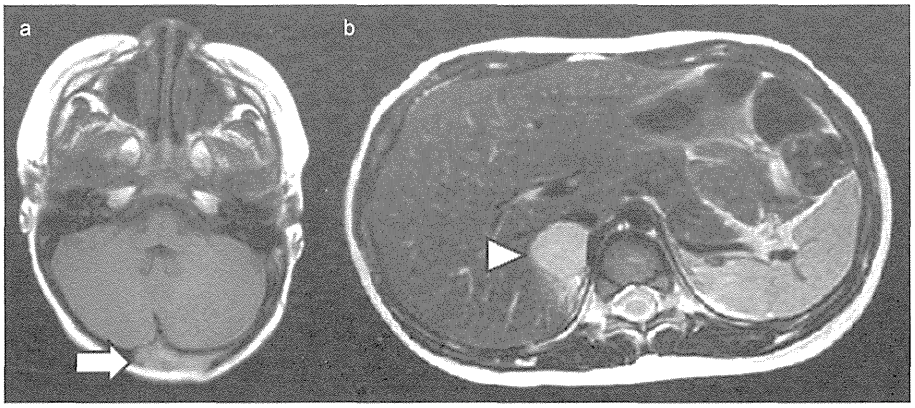

Fig. 1 (a) T1-weighted imaging of the head showing a solid tumor extending from the soft tissue of the occipital space to the intracranial space adjacent to the sagittal sinus (white arrow). (b) T1-weighted imaging of the abdomen showing the right adrenal tumor with T1-shortening (white arrowhead).

had hyperdiploid and non-amplified *MYCN*. A subsequent ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) scan, whole body CT, and bone marrow aspiration showed no metastasis; therefore, NB was classified as stage 1 on the International Neuroblastoma Stage System (INSS) and required no adjuvant therapy. In contrast, LCH required further treatment, because the tumor was too close to the sagittal sinus to be resected completely. She received chemotherapy consisting of oral prednisone, cytarabine, and vincristine according to the JLSG-02 protocol.

We ruled out congenital disease associated with tumorigenesis, including neurofibromatosis type 1, Gorlin syndrome, and Costello syndrome from the physical findings. Follow up found no relapse of either LCH or NB.

Discussion

We describe a 3-year-old girl with a history of KD and self-limiting cervical lymphadenopathy who presented with the synchronous occurrence of LCH and NB. The NB of the adrenal glands was asymptomatic and detected in the staging evaluation of LCH.

Both LCH and NB are rare diseases with incidences of 2–5 and 10.2 cases per million children, respectively.[1,3] The synchronous occurrence of LCH and NB, however, has occasionally been reported despite its rarity as calculated from the incidence of each disease (Table 1).[4–6] In three of four cases of synchronous occurrence of LCH and NB, both tumors were detected prior to the initial chemotherapy, which excluded the possibility of secondary malignancy in the origin.

The clinical behavior and outcome of NB vary widely depending on the histological and biological characteristics and localization. Additional chemotherapy was not necessary in the low-risk group, including INSS stage 1, even with incomplete resection, except in cases of organ dysfunction including spinal compression and liver infiltration. In contrast, multimodality therapy was necessary in the high-risk group. NB in the present case was classified as stage 1. Hence, considered retrospectively, even if the adrenal mass was not resected and diagnosed as NB, the outcome would not be exclusively worsened.

As shown in Table 1, NB was classified into stage 4 in two of four cases of synchronous occurrence of LCH and NB, which suggested that NB occurring with LCH could present unfavorable features, unlike those of the present case. If the present NB was classified into the intermediate or unfavorable group, the

Table 1 Clinical characteristics in co-occurrence of LCH and NB

	Fischer *et al.*[4]	Drozynska *et al.*[5]	Rayburg *et al.*[6]	Present case
Age (years)/Gender	2/M	9/M	5/M	3/F
Chief complaint	Polydipsia	Bone pain	Bone pain	Occipital mass
LCH location	Single system Multiple sites	Bone marrow	Single system Single site	Single system Single site
NB classification† (lesion site)	Stage 1 (left adrenal gland)	Stage 4 (left adrenal gland and multiple bone lesions)	Stage 4 (right adrenal gland and multiple bone lesions)	Stage 1 (right adrenal gland)
Elevation in sNSE/uHVA/uVMA	No/Yes/Yes	Yes/Yes/ND	ND/Yes/Yes	Yes/No/No
Treatment prior to the diagnosis of tumors	No	No	Surgery and chemotherapy for NB	No

†According to the International Neuroblastoma Staging System. LCH, Langerhans cell histiocytosis; NB, neuroblastoma; ND, no data; sNSE, serum neuron-specific enolase; uHVA, urinary homovanillic acid; uVMA, urinary vanillylmandelic acid.

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