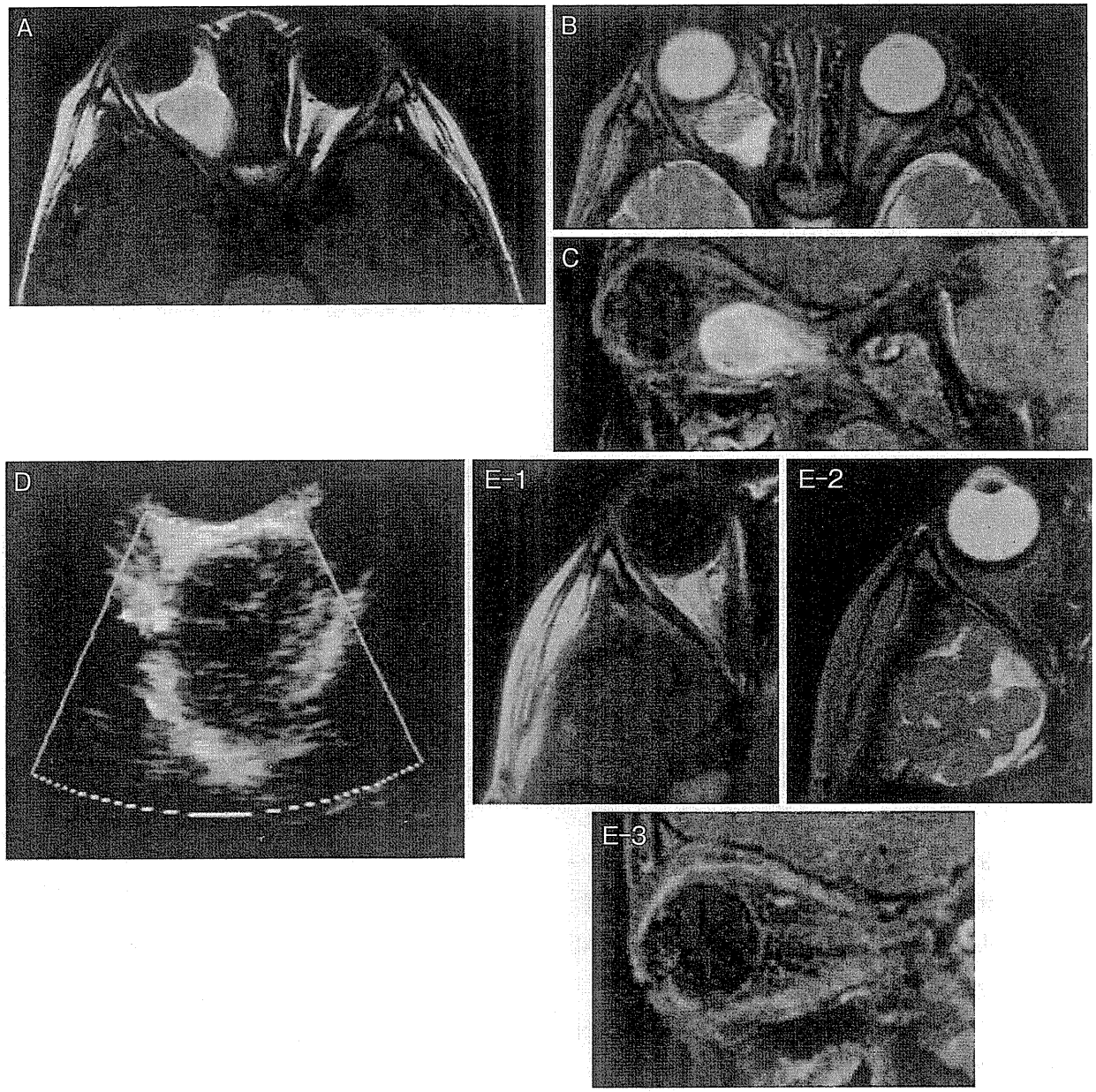

図 3　自然消失した眼窩内腫瘍

A：右眼窩内腫瘍(水平断 MRI，T1 強調)
初診時には右眼窩内原発の腫瘍。3.2 × 2.0 × 2.0 cm大。
右内側の眼窩壁を圧排している。
右眼球は前方に突出している。視神経と外眼筋を取り込んでいる。
B：右眼窩内腫瘍(水平断 MRI，T2 強調)
C：右眼窩内腫瘍(冠状断 MRI，TI 強調)
D：眼窩エコー検査(初診時)
辺縁に血流はあるが内部には明らかな血流を認めず，血管腫や栄養血管の存在は否定的である。
E：初診後 6 カ月後の MRI 検査
E-1；T1 強調水平断，E-2；T2 強調水平断，E-3；T1 強調矢状断。
腫瘤はほとんど消失している。

骨が写らないため病変部の広がりがわかりやすい。X線CT検査は隣接臓器，特に骨との関連がわかりやすい。シンチグラフィは転移巣，原発巣がわかりやすい。超音波は血流，内部性状が評価できるなどである。

眼窩は筋肉，神経，血管が複雑に入り組んでおりしかも狭くアプローチが難しい。生検術による合併症の危険性も高いため，各種の画像検査を組み合わせることでその腫瘍の性状，性質を分析し診断の補助とすることが重要である。

IV. 治療に関連した眼領域の合併症

強力な化学療法や放射線療法により薬剤性あるいは放射線による角膜・水晶体・硝子体・網膜・外眼筋などの眼合併症が種々認められる。小児固形腫瘍の治療中には定期的な眼科領域の診察が必要である。また治療により免疫能が抑制され，種々の感染症に罹患しやすくなるため，眼科領域の感染症についても注意が必要である。我々は髄芽腫の治療中に水痘帯状疱疹ウイルスの再活性化による急性網膜壊死を発症し，急激に片眼が失明した一男児例を経験した。眼科領域の合併症により治療が中断されることは原疾患の予後に密接に関連するため，早期発見早期治療が必要である。

おわりに

小児の固形腫瘍と眼科領域の関連について概説した。

小児の固形腫瘍では診断の契機となる症状には特徴的なものもあるが，実際には偶然発見されることも少なくない。「目をブランコにぶつけて瞼に紫斑ができた」，「サッカーボールがぶつかって膝が腫れた」，「おなかを跳び箱にぶつけて痛くなった」などと訴え受診し，精査により眼窩部横紋筋肉腫・骨肉腫・神経芽腫が発見された例を経験している。小児は訴えが少なく，保育者も気がつかないことも多い。

小児の固形腫瘍やその合併症は，早期に発見し速やかに治療を開始すれば予後も良好なものも多い。眼科領域は小児固形腫瘍と密接に関連する症状所見が多い。日頃から小児がんの存在を意識し，連携を密接にしていただければありがたい。

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Application of high-dose rate ^{60}Co remote after loading system for local recurrent neuroblastoma

Kiminobu Sugito [a,*], Takeshi Furuya [a], Hide Kaneda [a], Takayuki Masuko [a], Kensuke Ohashi [a], Mikiya Inoue [a], Taro Ikeda [a], Tsugumichi Koshinaga [a], Hiroshi Yagasaki [b], Hideo Mugishima [b], Toshiya Maebayashi [c]

[a]*Department of Pediatric Surgery, Nihon University School of Medicine, Tokyo 173-8610, Japan*
[b]*Department of Pediatrics, Nihon University School of Medicine, Tokyo 173-8610, Japan*
[c]*Department of Radiology, Nihon University School of Medicine, Tokyo 173-8610, Japan*

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Key words:
Remote after loading system;
Neuroblastoma

Abstract The local control of neuroblastoma is a very important treatment consideration. We describe a patient who received high-dose rate 60Co remote after loading system treatment for local control of recurrent neuroblastoma and discuss the efficacy of high-dose rate 60Co remote after loading system treatment.

Remote after loading system (RALS) treatment is brachytherapy using a high-dose rate (HDR) source delivered by remote control. The RALS treatment can accurately deliver a large amount of radiation to the cancer site and minimize the amount of normal tissue exposed to irradiation. Its use has been reported in various adult malignant tumors (cancer of the uterine cervix, esophageal cancer, breast cancer, prostate cancer, intraoral cancer, rectal cancer, bile duct cancer, etc); however, there are no reports of its effectiveness in pediatric solid tumors. The outcome for patients with recurrent neuroblastoma (NB) is extremely poor. Curative treatment of relapsed or resistant NB has not yet been established. We have confirmed that adjuvant radiotherapy has proven effective and is an important modality for local control in high-risk NB [1]. Here, we report a case in which HDR-60Co-RALS treatment is used for postoperative intraabdominal irradiation for local recurrence of NB between the right upper retroperitoneum and backside of the right hepatic lobe and discuss the efficacy of this modality. The hospital's institutional review board approved this study.

1. Case report

A 20-month-old boy was referred to our hospital because of abdominal distension. His family history was unremarkable. A mass was palpable in the right upper abdomen on physical examination and confirmed as a solid lesion on abdominal ultrasonography and computed tomography (Fig. 1). Metastases to bone and bone marrow were documented on scintigraphy. An open biopsy was obtained and was consistent

* Corresponding author. Tel.: +81 3 3972 8111x2452; fax: +81 3 3554 1321.
E-mail address: sugitou.kiminobu@nihon-u.ac.jp (K. Sugito).

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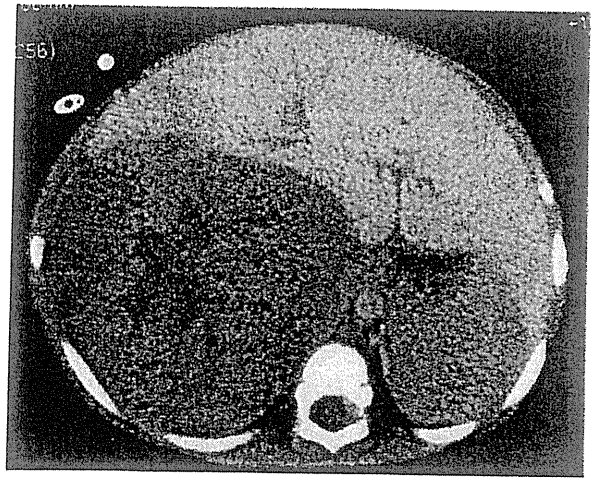

Fig. 1 Initial abdominal computed tomography scan showing a round encapsulated 10.7 × 8.0 cm solid mass.

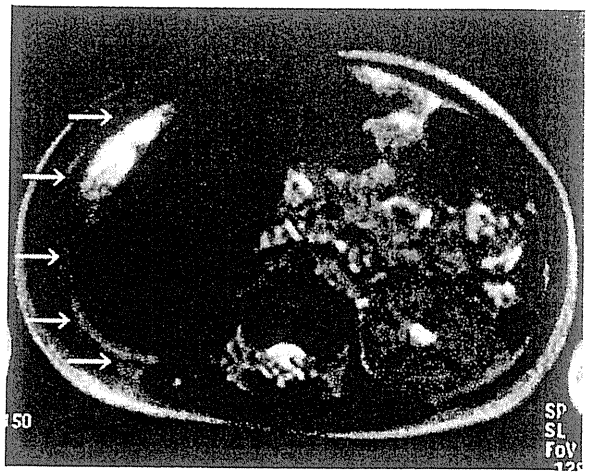

Fig. 2 T2-weighted abdominal magnetic resonance imaging showing a solid mass in the primary tumor field, which was outside the right hepatic lobe in the right upper retroperitoneum (arrows).

with NB stage 4, *MYCN* gene amplified with according to the International Neuroblastoma Staging System [2]. He received 5 courses of induction chemotherapy with a combination of cyclophosphamide, vincristine, etoposide, pirarubicine, and cisplatin or carboplatin in accordance with the protocol proposed by the Study Group of Japan for Advanced Neuroblastoma [3,4]. Before peripheral blood stem cell transplantation (PBSCT), he received high-dose chemotherapy (HDC; melphalan, etoposide, and carboplatin). After PBSCT, a complete gross total tumor resection was performed, and there were no viable tumor cells in the resected specimens on histological studies. Postoperatively, he received adjuvant radiation using a total dose of 21 Gy external beam radiation therapy (EBRT) to the final shrunken tumor field (after chemotherapy) and regional lymph node areas. He was discharged from the hospital in complete remission (CR). After 5 months in CR, the tumor markers vanillylmandelic acid and homovanillic acid in urine and serum neurone–specific enolase became elevated. T2-weighted abdominal magnetic resonance imaging confirmed recurrence of a solid mass in the primary tumor field outside the right hepatic lobe in the right upper retroperitoneum (Fig. 2). This was located beyond the reduced postoperative EBRT field and arose from the primary tumor field noted at initial presentation. We performed an open biopsy and obtained diagnosis of recurrent NB *MYCN* gene amplified without evidence of distant metastasis. We performed induction chemotherapy consisting of ifosfamide + etoposide × 2 courses, irinotecan × 2 courses, followed by 20 Gy EBRT against the partial lateral side of the recurrent tumor outside the liver. Further treatment included HDC (melphalan and busulfan) with PBSCT. At this time, it was difficult to secure the radiation field to the final shrunken tumor field. If EBRT or intraoperative radiation therapy (IORT) were used, we could not avoid total hepatic radiation. We performed a second tumor resection and inserted 4 plastic needles (outside diameter of 1.7 mm) percutaneously between the right upper retroperitoneum and the backside of the right hepatic lobe using intraoperative ultrasonography. These would be used postoperatively as applicators for irradiation. Pathologically, there were no viable tumor cells in the resected specimens. The Eckert & Ziegler BEBIG GmbH MultiSource HDR afterloader has a single 60Co source with nominal activity of 74 GBq. To plan treatment, HDRplus version 2.2 was used. The single-dose distribution was calculated to obtain a reference dose of 4 Gy (yellow line) at the unirradiated region (areas that had not been externally irradiated) or final shrunken recurrent tumor field (Fig. 3). Applications were done under intravenous anesthesia using pentazocine and midazolam. The electrocardiogram and O_2 saturation were monitored. All applicator insertions, radiograph generation, and treatments were performed in a dedicated brachytherapy suite equipped with imaging equipment. The total dose of 16 Gy (4 Gy per fraction, 4 fractions per 2 days) was delivered postoperatively. During RALS treatment, we used 2 antimicrobial drugs (meropenem and amikacin) to prevent infection. After the HDR-60Co-RALS treatment, the patient received HDC (cyclophosphamide, cylocide, and fludarabine) with cord blood stem cell transplantation and was discharged with a second CR. He has been followed up for 2 years and 2 months and has remained in CR after the secondary treatment with no evidence of recurrent disease.

2. Discussion

In NB, surgical resection followed by postoperative adjuvant radiotherapy has been the mainstay of treatment for the local control of the tumor. Insufficient dose delivery owing to the limitation of the radiation therapy technique is a major reason for local control failure. IORT has been described in a cohort of pediatric patients with varying benign conditions and malignancy [5]. External beam

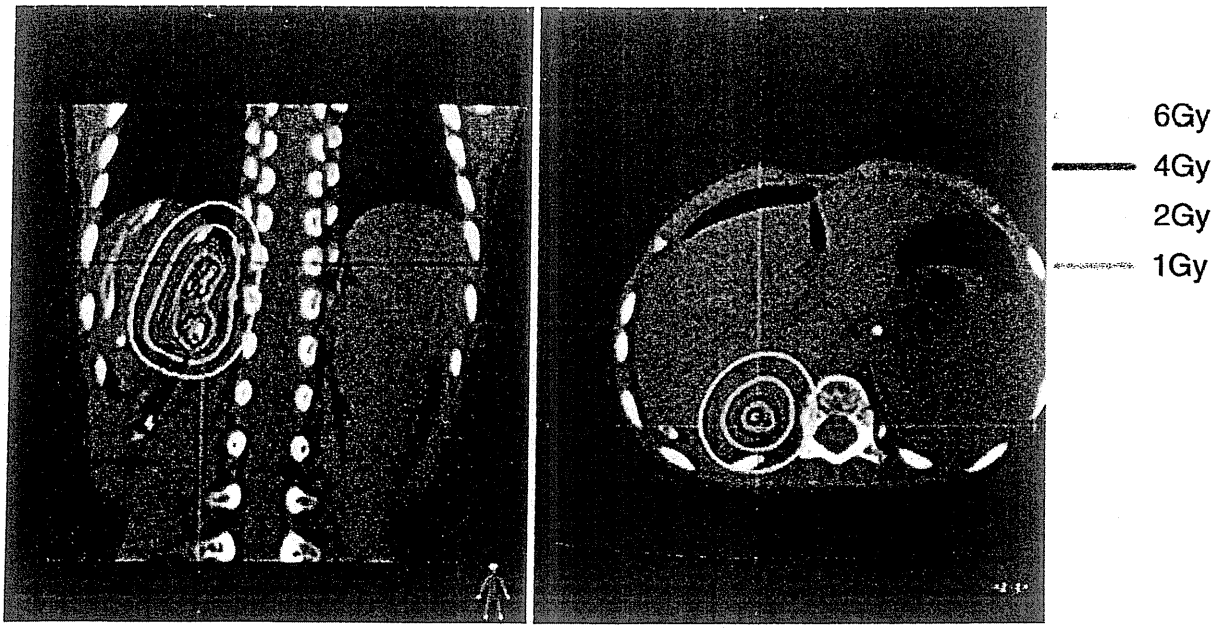

Fig. 3 The single-dose distribution was calculated to obtain a reference dose of 4 Gy at the unirradiated region (areas that had not been externally irradiated) or final shrunken recurrent tumor field.

radiation therapy also has been described as effective in cases of advanced NB [6-8]. The importance of intensive local treatment of advanced NB was emphasized in other studies [1,9-11]. The RALS treatment provides brachytherapy using the source of HDR and is a method to deliver the source into a patient's body by remote control. The HDR-RALS can correctly deliver a large amount of radiation to the cancer site and minimize the amount of normal tissue exposed to irradiation. These characteristics are similar to proton beam therapy. Considering growth and development and duration of radiation therapy, HDR-RALS treatment is very feasible for children. It appears that HDR-RALS treatment could be an alternate irradiation tool, along with IORT or EBRT, for the local control of NB. To the best our knowledge, this patient is the first case of NB to undergo intraabdominal irradiation using HDR-RALS.

Some investigators have attributed complications including episodes of gastrointestinal bleeding, gastrointestinal obstruction, colitis, hydronephrosis, neuropathy, scoliosis, increased infection, secondary neoplasms, and arterial stenosis to the use of IORT and EBRT [1,11-16]. Although we were concerned about the possible complications of HDR-RALS treatment to organs surrounding the applicators, such as the gastrointestinal tract, pancreas, liver, bile duct, abdominal nerves, and major vessels, there were no complications observed during the early postoperative period. Because of its physical characteristics, HDR-RALS treatment has a great advantage over conventional radiation therapy in delivering curative, high-dose irradiation without increasing the irradiation dosage to the surrounding critical organs. The HDR-RALS treatment is an advantageous method of treatment to ensure decreased radiation to other important organs while providing a curative dose to a target volume. The HDR-RALS treatment may be especially useful for locally advanced tumor patients with abdominal NB for whom it may be difficult to secure an IORT or EBRT field. The HDR-RALS treatment may also be effective for other pediatric tumors with radiation sensitivity.

The combination multimodal treatment (induction chemotherapy, EBRT, PBSCT, HDR-RALS treatment, and cord blood stem cell transplantation) may be an effective treatment strategy in salvage of recurrent high-risk NB. These observations suggest that further use of this therapeutic approach is warranted.

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Clinical Transplantation

Feasibility of tacrolimus, methotrexate, and prednisolone as a graft-versus-host disease prophylaxis in non-T-cell-depleted haploidentical hematopoietic stem cell transplantation for children

Mochizuki K, Kikuta A, Ito M, Sano H, Akaihata M, Kobayashi S, Ohto H, Hosoya M. Feasibility of tacrolimus, methotrexate, and prednisolone as a graft-versus-host disease prophylaxis in non-T-cell-depleted haploidentical hematopoietic stem cell transplantation for children. Clin Transplant 2011: 25: 892–897. © 2010 John Wiley & Sons A/S.

Abstract: In this study, we evaluated the feasibility of our graft-versus-host disease (GVHD) prophylaxis with tacrolimus, methotrexate, and prednisolone in non-T-cell-depleted haploidentical hematopoietic stem cell transplantation (HSCT) for children. Twenty-one consecutive patients including those with hematological malignancies (n = 11), solid tumors (n = 7), and non-malignancies (n = 3) were analyzed. Myeloablative and reduced intensity conditionings were carried out in 5 and 16 patients, respectively, and both of the regimens contained anti-human T-lymphocyte immunoglobulin. Twenty (95%) of the 21 patients achieved primary engraftment. Acute GVHD of grades II–IV and III–IV were observed in nine (47%) and one (5%) patient, respectively, all of which were controllable by steroids. Chronic GVHD was observed in eight (51%) of the 17 evaluable patients, and one of them developed steroid refractory chronic GVHD. Treatment-related mortality occurred in three patients (15%), as a result of acute pancreatitis, chronic GVHD, and EB virus associated lymphoproliferative disease. The median follow-up of the 13 survivors was 24 months, and the two-yr probability of overall survival was 68%. The Karnofsky performance scale score of the 13 survivors was 100%. These results indicated the feasibility of our GVHD prophylaxis in non-T-cell-depleted haploidentical HSCT for children.

Kazuhiro Mochizuki[a], Atsushi Kikuta[b], Masaki Ito[a], Hideki Sano[a], Mitsuko Akaihata[a], Shogo Kobayashi[a], Hitoshi Ohto[c] and Mitsuaki Hosoya[a]

[a]Department of Pediatrics, Fukushima Medical University School of Medicine, [b]Division of Pediatric Oncology, Fukushima Medical University Cancer Center and [c]Department of Blood Transfusion and Transplantation Immunology, Fukushima Medical University Hospital, Fukushima, Japan

Key words: children – graft-versus-host disease – haploidentical – non-T-cell-depleted – stem cell transplantation

Conflict of interest: The authors declare that they have no conflicts of interest in relation to this manuscript.

Corresponding author: Atsushi Kikuta, MD, PhD, Division of Pediatric Oncology, Fukushima Medical University Cancer Center, 1 Hikariga-oka, Fukushima City, Fukushima 960-1295, Japan. Tel.: +81 24 547 1295; fax: +81 24 548 6578; e-mail: akikuta@fmu.ac.jp

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Allogeneic hematopoietic stem cell transplantation (HSCT) has been successfully carried out to treat many malignant and non-malignant diseases. However, more than 50% of the patients requiring HSCT are unable to find a suitable adult stem cell donor in a timely fashion. On the other hand, haploidentical HSCT from family members provides an option for nearly all the patients lacking a compatible donor (1, 2). However, the usefulness of this approach is often limited by significant rates of graft-versus-host disease (GVHD), graft rejection, and other complications. Lu et al. (3) reported a novel method of non-T-cell-depleted haploidentical HSCT in which anti-human T-lymphocyte immunoglobulin (ATG) was included in the conditioning regimen. They used cyclosporine A (CsA), methotrexate (MTX), and mycophenolate mofetil (MMF) as a GVHD prophylaxis and showed survival, relapse, treatment-related mortality (TRM), and GVHD outcomes comparable to those of transplantations from HLA-identical siblings. Recently, other groups have also reported

the usefulness of non-T-cell-depleted haploidentical HSCT, but an optimal regimen for GVHD prophylaxis has not yet been determined (2, 4, 5). Since 2003, we have been using tacrolimus, MTX, and prednisolone (PSL) as a GVHD prophylaxis in the ATG containing non-T-cell-depleted haploidentical HSCT. In this study, we evaluated the feasibility of our GVHD prophylaxis in the non-T-cell-depleted haploidentical HSCT for children.

Patients and methods

Patients

Twenty-one consecutive children who received non-T-cell-depleted HLA-haploidentical HSCT from a family donor between September 2004 and December 2009 at Fukushima Medical University Hospital were retrospectively analyzed (Table 1). Eleven patients had hematological malignancies, seven had solid tumors, and the remaining three patients had non-malignancies. In the patients with refractory disease, haploidentical family donors were chosen owing to the anticipation of stronger graft-versus-tumor effect. On the other hand, in the patients without refractory disease or non-malignancies, haploidentical HSCT was performed because there was no available HLA-identical-related or unrelated donor. Of the 11 patients with hematological malignancies, two were not in remission (Nos. 3 and 20), while all the patients with solid tumors were in primary refractory or relapsed phases. The institutional review board approved the protocol, and written informed consent was obtained from the patients or their guardians and family donors. Follow-up for all the patients was continued through August 2010.

Donor source, HLA disparity, and stem cell graft

Donors included fathers (9), mothers (9), and siblings (3). HLA-A, HLA-B, HLA-C, and HLA-DRB1 typing was performed by intermediate-resolution DNA typing (Genosearch HLA; MBL, Nagoya, Japan) (Table 1). HLA disparities in both graft-versus-host and host-versus-graft directions included two loci mismatches in one case, three loci mismatches in five cases, and four loci mismatches in the other 15 cases. None of the donors or recipients had anti-HLA antibodies against the mismatched antigens between each donor and recipient.

Eighteen patients received bone marrow as a stem cell source. Recently, the evidence regarding GVHD after haploidentical HSCT, which is mostly controllable by using ATG and intensive GVHD prophylaxis, has led us to change the stem cell source from bone marrow to mobilized peripheral blood stem cells (Nos. 17, 20, and 21).

Conditioning regimen and GVHD prophylaxis

Myeloablative and reduced intensity conditioning (RIC) were carried out in 5 and 16 patients, respectively, and both of the regimens contained ATG (Table 1). The ATG product that we used before March 2009 was Zetbulin (Nihon Zouki, Tokyo, Japan) (total 10 mg/kg). It was then replaced by thymoglobulin (Genzyme Japan, Tokyo, Japan) because this has been covered by health insurance since April 2009 in Japan. The initial total dose of thymoglobulin was 10 mg/kg of recipient's body weight according to the manufacturer's instructions for HSCT conditioning. However, because of adverse events mainly because of viral infections, the total doses of thymoglobulin were gradually reduced to 2.5 mg/kg. In this study, there were four cases with primary graft failure of the first bone marrow transplantation or cord blood transplantation (CBT). They received haploidentical second (Nos. 11, 12, and 13) or third (No. 7) HSCT after very short-term conditioning with fludarabine and ATG. Three of them (Nos. 11, 12, and 13) received a myeloablative conditioning in their initial HSCT.

The GVHD prophylaxis was conducted with tacrolimus, short-term administration of MTX, and PSL. Tacrolimus was started on day −1, which was continuously administered intravenously. The concentration of tacrolimus in peripheral blood was adjusted between 10 and 15 ng/mL. Three or four wk after transplantation, the tacrolimus administration was changed to the oral route with the trough level targeted at 5–10 ng/mL. MTX (10 mg/m^2) was administered intravenously on day +1, and then 7 mg/m^2 was administered on days +3 and +6 after transplantation. PSL was begun on day +0 with an initial dose of 1 mg/kg/d. When there was no sign of acute GVHD, from day +29, the PSL dose was tapered every week and was discontinued within two and six months after transplantation in the patients with malignant and non-malignant diseases, respectively.

Statistics

The severities of acute and chronic GVHD were diagnosed using standard criteria and the NIH criteria (6, 7). Transplantation-related toxicities were evaluated using the common terminology criteria for adverse events (CTCAE version 4.0; http://evs.nci.nih.gov/ftp1/CTCAE/About.html)

Table 1. Patient/donor characteristics, HLA disparity, stem cells and conditioning regimen

No	Age	Gender	Disease	Status	Donor	HLA disparity (A, B, C, DRB1)	Stem cell source and dose (per kg) ANC ($\times 10^8$) or [a]CD34 ($\times 10^6$)		Conditioning regimen
1	2–3	M	CGD	With systemic infection	Mother	4/8	BM	5.7	Flu (150 mg/m^2) + Mel (140 mg/m^2) + ATG[b] (20 mg/kg)
2	5–9	M	Neuroblastoma	1 relapse	Sibling	4/8	BM	4.8	Flu (150 mg/m^2) + TEPA (800 mg/m^2) + ATG[b] (20 mg/kg)
3	19–0	M	CML	Accelerated phase	Father	4/8	BM	4.1	Flu (150 mg/m^2) + Mel (140 mg/m^2) + BU (8 mg/kg) + CY (50 mg/kg) + ATG[b] (20 mg/kg)
4	5–6	M	AML (M2)	2 CR	Mother	4/8	BM	7.3	Flu (150 mg/m^2) + Mel (140 mg/m^2) + ATG[b] (10 mg/kg)
5	10–5	M	Fanconi anemia	Post IST	Mother	4/8	BM	4.8	TLI (3) + Flu (180 mg/m^2) + CY (40 mg/kg)) + ATG[b] (10 mg/kg)
6	4–9	M	Wilms tumor	Primary refractory	Father	4/8	BM	3.9	Flu (180 mg/m^2) + Mel (140 mg/m^2) + TEPA (400 mg/m^2) + ATG[b] (10 mg/kg)
7	6–3	M	ALL	2 CR, GF after uBMT and uCBT	Mother	4/8	BM	5.0	Flu (90 mg/m^2) + ATG[b] (15 mg/kg)
8	17–10	M	Rhabdomyosarcoma	1 relapse	Father	5/8	BM	3.1	Flu (150 mg/m^2) + Mel (140 mg/m^2) + ATG[b] (10 mg/kg)
9	9–5	M	ALL	2 CR	Mother	4/8	BM	4.9	TBI (12) + VP16 (60 mg/kg) + CY (120 mg/kg) + ATG[b] (10 mg/kg)
10	13–4	M	Neuroblastoma	Primary refractory	Sibling	4/8	BM	2.6	Flu (150 mg/m^2) + Mel (140 mg/m^2) + ATG[b] (10 mg/kg)
11	11–0	F	ALL	2 CR, GF after uCBT	Father	4/8	BM	5.4	Flu (150 mg/m^2) + Mel (140 mg/m^2) + ATG[b] (10 mg/kg)
12	3–1	F	EBV-PTCL	Chronic phase, GF after uCBT	Mother	4/8	BM	8.5	TBI (3) + Flu (150 mg/m^2) + Mel (140 mg/m^2) + ATG[b] (10 mg/kg)
13	14–4	M	AML (M0; FLT3-ITD)	1 CR, GF after uCBT	Father	4/8	BM	4.4	Flu (150 mg/m^2) + Mel (140 mg/m^2) + ATG[b] (10 mg/kg)
14	5–7	F	Neuroblastoma	1 relapse	Mother	4/8	BM	7.0	Flu (150 mg/m^2) + Mel (140 mg/m^2) + ATG[b] (10 mg/kg)
15	4–2	F	NK cell lymphoma	2 CR, post-uCBT	Mother	5/8	BM	7.4	Flu (150 mg/m^2) + Mel (140 mg/m^2) + ATG[c] (10 mg/kg)
16	10–0	F	Neuroblastoma	1 relapse	Father	5/8	BM	8.2	Flu (150 mg/m^2) + Mel (140 mg/m^2) + ATG[c] (10 mg/kg)
17	19–10	M	CGD	With systemic infection	Sibling	6/8	PBSC	[a]4.7	TBI (3) + Flu (150 mg/m^2) + Mel (140 mg/m^2) + ATG[c] (5 mg/kg)
18	4–10	M	t-AML (M4)	1 CR	Father	4/8	BM	7.3	TBI (12) + VP16 (60 mg/kg) + CY (120 mg/kg) + ATG[c] (5 mg/kg)
19	18–10	F	Mesenchymal chondrosarcoma	1 relapse	Mother	5/8	BM	2.8	Flu (150 mg/m^2) + Mel (140 mg/m^2) + ATG[c] (2.5 mg/kg)
20	7–8	M	AML (M2)	1 relapse	Father	5/8	PBSC	[a]8.4	TBI (12) + Ara-C (12 g/m^2) + CY (120 mg/kg) + G-CSF (5 μg/kg/d for 2 d) + ATG[c] (2.5 mg/kg)
21	10–11	F	ALL	2 CR	Father	4/8	PBSC	[a]7.4	TBI (12) + VP16 (60 mg/kg) + CY (120 mg/kg) + ATG[c] (2.5 mg/kg)

[a]CD34 positive cells. Age: year–months, CGD, chronic granulomatous disease; EBV-PTCL, EBV assosiated peripheral T cell lymphoma; CBT, cord blood transplantation; GF, graft failure; IST, immunosuppressive therapy; Flu, fludarabine; Mel, melphalan; TESPA, thio-TEPA; ATG, anti-human T-lymphocyte immunoglobulin; VP16, etoposide; M, male; F, female; u, unrelated.
[b]Zetbulin.
[c]Thymoglobulin.

Table 2. Engraftment, GVHD, complications and outcome

No	Engraftment (d)	Acute GVHD grade and stage (skin, liver, GI)	Chronic GVHD severity (organ: NIH score)	Other complications within 100 d	Follow up (months)	Outcome, KPS score of survivors
1	15[a]	G0	NE	CMV reactivation	71	Alive without disease (re-CBT after secondary GF), 100%
2	19	G0	NE	Acute pancreatitis	1	Dead (acute pancreatitis)
3	Not achieved	NE	NE	NO	7	Dead (acute GVHD after re-CBT)
4	13	G0	Mild (skin: 1)	NO	54	Dead (BM relapse at day135, MOF after re-HSCT)
5	14	G0	Mild (mouth: 1)	RPLES, hemorrhagic cyctitis, CMV reactivation	57	Alive without disease, 100%
6	14	G0	NO	CMV reactivation, zoster	9	Dead (disease progression)
7	15	G2 (3, 1, 1)	Severe (PS: 3, skin: 3, mouth: 1, GI: 1, lungs: 2)[b]	Sepsis	48	Dead (chronic GVHD)
8	16	G2 (3, 0, 1)	NO	NO	9	Dead (disease progression)
9	16	G2 (3, 0, 0)	Severe (skin: 3, mouth: 1)	NO	41	Alive (BM relapse at day 472), 100%
10	16	G0	Moderate (mouth: 2, eyes: 2)	Zoster	29	Alive (disease progression), 100%
11	15	G2 (2, 0, 1)	Severe (skin: 3, mouth: 1, eyes: 1)	Zoster	29	Alive without relapse, 100%
12	20	G2 (3, 0, 0)[c]	Moderate (skin: 2, mouth: 1)	Abcess (neck), EBV reactivation, CMV reactivation	27	Alive without relapse, 100%
13	15	G0	NO	NO	24	Alive without relapse, 100%
14	16	G0	NO	NO	12	Dead (disease progression)
15	15	G0	NE	EBV-LPD	2	Dead (EBV-LPD)
16	15	G0	Moderate (mouth: 1, GI: 2)	NO	15	Alive without disease progression, 100%
17	15	G1 (2, 0, 0)[c]	NO	CMV reactivation	13	Alive without disease, 100%
18	14	G2 (3, 0, 1)	NO	Abcess (penis), CMV reactivation	12	Alive without relapse, 100%
19	12	G3 (3, 0, 4)[d]	NO	IPS, avascular necrosis of the femoral head	11	Alive without disease progression, 100%
20	15	G2 (3, 0, 1)[d]	NO	CMV reactivation	10	Alive without relapse, 100%
21	12	G2 (3, 0, 0)[d]	NO	NO	8	Alive without relapse, 100%

NE, not evaluated; NO, not observed; GI, gastrointestinal tract; PS, performance status; RPLES, reversible posterior leukoencephalopathy syndrome; LPD, lymphoproliferative disease; KPS, Karnofsky performance scale; CBT, cord blood transplantation; CMV, cytomegalovirus; MOF, multiple organ failure; HSCT, hematopoietic stem cell transplantation; GF, graft failure; GVHD, graft-versus-host disease; IPS, idiopathic pneumonia syndrome.
[a]Secondary graft failure.
[b]Steroid refractory.
[c]Late onset acute GVHD.
[d]Recurrence of acute GVHD.

presented by the National Cancer Institute. TRM was defined as death during continuous post-transplantation remission. The cumulative incidence of GVHD and provability of overall survival were estimated using the Kaplan–Meier method.

Results

Engraftment

Twenty (95%) of the 21 patients achieved primary engraftment with the median time of neutrophil recovery of 15 d (range: 12–20 d) (Table 2). Among them, one patient with chronic granulomatous disease (No. 1) experienced secondary graft failure, 73 d after haploidentical HSCT. The platelet engraftment was evaluated in 20 patients who survived longer than 50 d, and 19 patients (95%) met the criteria of platelet recovery with the median time of 33 d (range: 22–45 d). Although there were three urgent cases (Nos. 7, 11, and 13) as the result of primary graft failure of CBT, all of them achieved engraftment after haploidentical re-HSCT. Moreover, all the patients who achieved engraftment established complete donor chimerism by day +30 except one who experienced secondary graft failure.

GVHD

Acute GVHD was evaluated in 20 patients who achieved primary engraftment, and those of grades

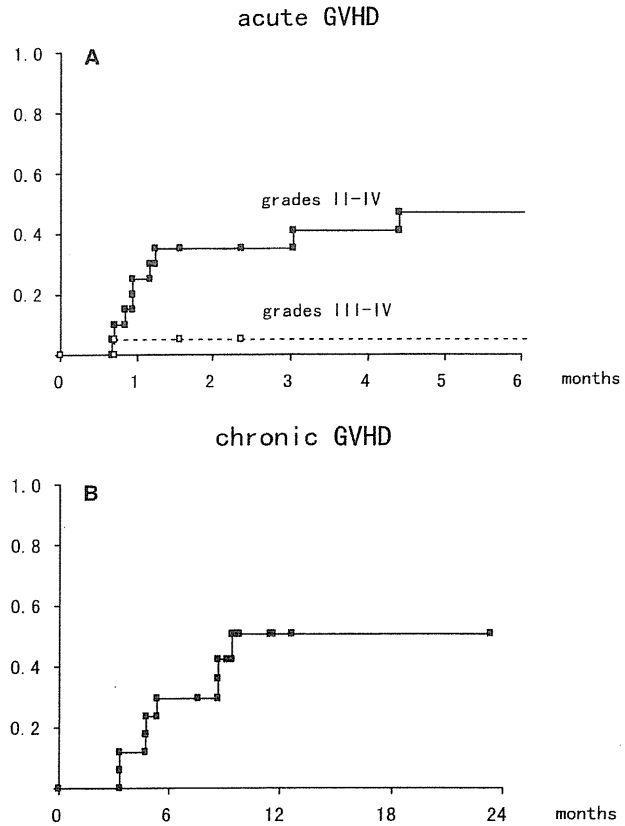

Fig. 1. (A) Cumulative incidence of acute graft-versus-host disease (GVHD) after (n = 20). Solid line indicates acute GVHD of grade II–IV, and dotted line indicates that of grade III–IV. (B) Cumulative incidence of chronic GVHD (n = 17). Eight of the 17 patients who survived longer than 100 d after non-T-cell-depleted haploidentical hematopoietic stem cell transplantation developed chronic GVHD.

I, II, and III occurred in one, eight, and one patient, respectively (Fig. 1a, Table 2). No patient developed acute GVHD of grade IV in this study. Clinical manifestations included skin rash in nine (90%), severe diarrhea in six (60%), and hepatic dysfunction in one (10%), all of which responded to steroids: temporary augmentation of PSL (1–2 mg/kg/d) (Nos. 7, 9, 11, 12, 18, and 21), methyl PSL pulse therapies (Nos. 8, 19, and 20), or oral administration of beclomethasone dipropionate (Nos. 20 and 21).

Chronic GVHD was evaluated in 17 patients who achieved engraftment and survived longer than 100 d after transplantation, and it was observed in eight patients (51%) (Fig. 1b). One patient (No. 7) who received haploidentical HSCT as his third transplantation, developed steroid refractory chronic GVHD. The other patients' symptoms of chronic GVHD were mainly skin or mouth lesions, which were manageable with PSL and tacrolimus.

Complications

One patient (No. 5) developed reversible posterior leukoencephalopathy syndrome, and another patient (No. 19) developed idiopathic pneumonia syndrome, both of which recovered following conventional treatment (Table 2). The latter patient also developed avascular necrosis of the femoral head on both sides. Infectious complications including cytomegalovirus (CMV) antigenemia (n = 7), temporary elevation of EBV-DNA (n = 1), EBV-lymphoproliferative disease (EBV-LPD) (n = 1), zoster (n = 3), hemorrhagic cystitis because of BK virus (n = 1), and severe bacterial infections (n = 3) were observed within 100 d after transplantation. These infectious complications, except one case of EBV-LPD, were controllable by using conventional anti-microorganism therapies or decreasing immune suppression. Other grade III to IV toxicities observed within 100 d after transplantation were as follows: grade III oral mucositis in five patients, grade III hypertension in four patients, and grade IV hyperglycemia in another patient. TRM occurred in three patients (15%) as a result of acute pancreatitis (No. 2), chronic GVHD (No. 7), and EBV-LPD (No. 15).

Survival

The median follow-up of the 13 survivors was 24 months (range: 8–71 months). The two-yr probability of overall survival in this study was 68%. The quality of life of all 13 survivors was evaluated to be 100% according to the Karnofsky performance scale (KPS).

Discussion

Allogeneic HSCT is the only curative approach for a number of patients with malignant or non-malignant diseases. In this study, we showed the feasibility of our GVHD prophylaxis in non-T-cell-depleted haploidentical HSCT for children. Concerning engraftment after haploidentical HSCT, an allograft needs to traverse HLA-mismatched barriers. According to other clinical studies, high engraftment rates (96–100%) were reported in non-T-cell-depleted haploidentical HSCT (3–5, 8). In this study of pediatric patients, 95% of the recipients achieved primary engraftment, which was comparable to previous reports. Moreover, 11 of the 13 patients who received RIC at the initial HSCT achieved long-term engraftment. In particular, high engraftment rate can be expected for patients who had received several courses of

chemotherapies prior to the haploidentical RIC-HSCT.

One of the major problems associated with the non-T-cell-depleted haploidentical HSCT is severe GVHD. According to Liu et al. (9), in their series of haploidentical HSCT without *in vitro* T-cell depletion, by using ATG (10 mg/kg) (Thymoglobulin; Genzyme, Cambridge, MA, USA) in the conditioning regimen and CsA + MTX + MMF as the GVHD prophylaxis, the cumulative incidences of acute GVHD of grades II–IV, III–IV, and that of chronic GVHD were 57.2%, 13.8%, and 52.7%, respectively. In this study, nearly half of the patients developed acute GVHD of grade II or III, but no patient developed that of grade IV, and all the symptoms of acute GVHD were controllable by steroids. On the other hand, TRM associated with chronic GVHD occurred in one case, while most of the other patient's symptoms of chronic GVHD were manageable with PSL or tacrolimus. Therefore, we believe that our GVHD prophylaxis, which includes tacrolimus, MTX, and PSL, suppresses the incidence of severe acute and chronic GVHD within an acceptable range after ATG containing non-T-cell-depleted haploidentical HSCT.

The probability of infectious complications after non-T-cell-depleted haploidentical HSCT was considered to be high because intensive immune suppressive regimens for GVHD prophylaxis are usually adopted. In this study, the incidence of viral infections, especially CMV reactivation, was higher than that in HSCT from HLA-identical donors. However, most of these complications were manageable by conventional anti-viral treatment or decreasing immune suppression. The high incidence of viral infections after haploidentical HSCT might be owing to the administration of ATG in the conditioning regimen; and therefore, an optimal dose of ATG needs to be determined carefully.

The reported incidence of TRM after HSCT from matched related, matched unrelated, or cord blood were about 20–30% (10–12). In this study, TRM occurred in three patients (15%), which were thought to be within an acceptable range because more than one-third of the patients had complications prior to HSCT. Moreover, the KPS scores of the survivors were all 100%, and most of them have obtained an excellent prognosis. The results described here indicate the feasibility of our GVHD prophylaxis with tacrolimus, MTX, and PSL in non-T-cell-depleted haploidentical HSCT for children. Further clinical studies are warranted to determine the effectiveness of this approach.

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Is the prognosis of stage 4s neuroblastoma in patients 12 months of age and older really excellent?

Tomoko Iehara [a,*], Eiso Hiyama [b], Tatsuro Tajiri [c], Akihiro Yoneda [d], Minoru Hamazaki [e], Masahiro Fukuzawa [f], Hajime Hosoi [a], Tohru Sugimoto [a], Tadashi Sawada [a]

[a] *Department of Pediatrics, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kawaramachi-Hirokoji Kamigyo-ku, Kyoto 602-8566, Japan*
[b] *Natural Science Center for Basic Research and Development, Hiroshima University, Hiroshima 734-8551, Japan*
[c] *Department of Pediatric Surgery, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan*
[d] *Department of Pediatric Surgery, Osaka Prefectural Hospital Organization, Osaka 594-0011, Japan*
[e] *Depatment of Pathology, Shizuoka Children's Hospital, Shizuoka 420-8660, Japan*
[f] *Department of Pediatric Surgery, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan*

KEYWORDS
Neuroblastoma
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Abstract *Purpose:* In the International Neuroblastoma Risk Group (INRG) classification system, stage 4s was changed into stage MS in children less than 18 months of age. Stage MS is defined as a metastatic disease with skin, liver and bone marrow, similar to INSS stage 4s. To evaluate the outcome of stage 4s cases in patients 12 months of age and over and to determine the appropriate treatment strategy.
Method: We performed a retrospective review of 3834 patients registered with the Japanese Society of Pediatric Oncology and Japanese Society of Pediatric Surgeons between 1980 and 1998.
Results: The rates of stage 4s patients were 10.7%, 6.3% and 3.3% in patients of $\leqslant$11 months of age, from $\geqslant$12 to $\leqslant$17 months of age, $\geqslant$18 months of age, respectively. The 5 year event-free survival rates were 89.4%, 100% and 53.1%, respectively. The rates of *MYCN* amplification and unfavourable histology were smaller in stage 4s groups than stage 4 groups in all ages.
Conclusion: In the children 12 months of age and older, stage 4s cases are markedly different from stage 4 cases in regard to the clinical features and prognosis. The prognosis of stage 4s cases from $\geqslant$12 to $\leqslant$17 months of age is excellent. The concept of stage MS appears to be appropriate.

* *Corresponding author:* Tel.: +81 75 251 5571; fax: +81 75 252 1399.
E-mail address: iehara@koto.kpu-m.ac.jp (T. Iehara).

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1. Introduction

Neuroblastoma is the most common extra cranial solid tumour in childhood. The prognosis of the disease is largely dependent on the age of the child and the extension of the tumour at diagnosis. In general, the prognosis for neuroblastoma in infants is much more favourable than in older children. Stage 4s neuroblastoma, first described by Evans, is a special metastatic disease for patients <12-months-old associated with a favourable prognosis.[1,2] Although these patients have wide metastatic disease, they have a favourable prognosis and also have high rates of a spontaneous regression. The stage 4s neuroblastoma is defined as an infant <12-months-old with metastases restricted to the liver, skin, and/or bone marrow, in which the primary tumour is localised (stage 1 or 2).

Recently, the International Neuroblastoma Risk Group (INRG) classification system was developed in order to establish a consensus approach for pre-treatment risk stratification. The new International Neuroblastoma Risk Group Staging System (INRGSS) was developed for the INRG.[3,4] To classify neuroblastoma patients by INRG classification system, we used the criteria of INRG stage, age, histological category, grade of tumour differentiation, *MYCN* status, 11q aberrations and tumour cell ploidy. In this INRG system, stage 4s changes to stage MS in children <18 months old. Stage MS is defined as a metastatic disease with special features, similar to INSS stage 4s, although there is no restriction regarding the size of the primary tumour. The metastases are restricted to the skin, liver and bone marrow. Age is not a component of the definition of stage MS.[3,4] Therefore, stage MS includes children aged from more than 12 months to less than 18 months of age. Conventionally, an age of ⩾12 months has been the reference point for decisions for stage 4 neuroblastoma. Recently, an age cutoff of 18 months was proposed in a large-scale research study.[5]

The present study was undertaken to clarify how high the frequency of the stage 4s cases of ⩾12 months and to clarify whether the prognosis is excellent or not for a decision-appropriate treatment strategy. This is the first report of stage 4s neuroblastoma in patients ⩾12 months of age.

2. Patients and method

A retrospective review of 3834 patients with neuroblastoma was performed. The patients were registered with the Committee of Neuroblastoma in the Japanese Society of Pediatric Oncology and Japanese Society of Pediatric Surgeons between 1980 and 1998.

The patients were divided into three groups: ⩽11-months of age, ⩾12 to ⩽17-months of age and ⩾18-months of age.

From these three groups, we extracted the cases suited for stage 4s. The cases of metastasis were limited to the skin, liver or bone marrow. The primary tumours were observed within the tumour capsule (C_1) or outside the tumour capsule but not beyond the midline (C_2) and without contra lateral regional lymph node, in other words, the tumour of stage 3 was omitted. The maximum diameter of the primary tumours is less than 10 cm. In infancy, the stage 4s definition excluded bone marrow metastasis with more than 10% tumour cell infiltration. However, in this study, the ratio of the infiltration tumour cells is not considered in the stage 4s cases >12 months old. Thereafter, we examined the frequency of these extracted cases and compared the clinical feature and prognosis of stage 4 with those of 4s cases.

The stage 4s cases <12 months old were given either six cycles of the low-dose regimen, consisted of a low-dose of cyclophosphamide and vincristine over a 2-week period to shrink the tumour, followed by surgical resection. Stage 4 cases were treated with intensive chemotherapy consisting of cyclophosphamide and pirarubicin, cisplatin, vincristine or etoposide. Infants less than 12-months old were treated with reduced dosages. After 1992, many cases, especially cases with *MYCN* amplification, received high-dose chemotherapy with stem cell transplantation.

Amplification of the *MYCN* had been studied in children with those tumours since 1990 in JAPAN.

The histology of the primary tumour 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 since 1994.

2.1. Statistical analysis

The Kaplan and Meier product limit methods were used to estimate the event-free survival (EFS) and the over-all survival (OS). The EFS calculated from diagnosis to the first event; relapse, progression or death (exception of other reason death). OS is calculated from diagnosis to death, excluded other reason death. Because the number of the events of each group was very small, we omitted the other reason death not to make bias. The Cox proportional hazards model was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CI).The exact test from the permutation of the log-rank statistic was used to compare the EFS or OS probabilities between subgroups of patients. Differences between the two groups in categorical data were analysed by means of Fisher's exact probability test or the chi-square test. Two-sided *P*-values under 0.05 were considered as significant.

3. Results

The rates of stage 4s patients were 10.7%, 6.3% and 3.3% in patients ⩽11-months of age, 12–17 months of age, and ⩾18 months of age, respectively.

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Stage 4s patients frequently present even at 12 months of age and older, although their frequency decreases with age. Since 1985, the high numbers of patients under 11 months of age is the reason why cases detected by screening are included. However, there is no difference in the frequency of patients detected by screening between stage 4 and 4s groups (Table 1).

The rates of *MYCN* amplified stage 4s patients were 3.7%, 0% and 0% in those ⩾11 months of age, 12–17-months of age and ⩾18 months of age, respectively. The rates of *MYCN* amplified patients were smaller in the stage 4s groups than in the stage 4 groups in each group ($P < 0.001$, $P = 0.04$ and $P < 0.001$, respectively). Similarly, the rates of patients with unfavourable histology were smaller in the stage 4s groups than in the stage 4 groups. However, the difference in the frequency of patients with unfavourable histology between the two stage groups was not significant in those 12–17-months of age, because of the small number of patients. The stage 4s patients displayed a lower mean serum LDH value than the stage 4 patients in each group (Table 1).

In stage 4s patients ⩽11-months old, observation and surgery alone were 6.2% and 6.9%, respectively. Infants less than 12-months old were treated with a different protocol between stage 4s and 4 group. Those stage 4s patients received less dose chemotherapy than stage 4. Patients >12-months of age with stage 4s and 4 tumour received the same induction chemotherapy and most of them received surgical resection. The other hand, the number of patients who received high-dose chemotherapy with stem cell transplantation were smaller in the stage 4s groups than in the stage 4 groups in each age category ($P = 0.002$ and $P = 0.017$, respectively), for patients ⩽11 months of age and 12–17-months of age. All patients ⩽11-months of age who received high dose chemotherapy have tumours with *MYCN* amplification. There are no patients who received surgical resection only or

Table 1
Characteristics of patients with INSS stage 4 or stage 4s neuroblastoma.

		⩽11 m			12–17 m			⩽18 m		
		No.	%	*P*	No.	%	*P*	No.	%	*P*
Patients	Total	2579			252			1003		
	Stage 4s	275	10.7		16	6.3		33	3.3	
	Stage 4	294	11.4		73	29.0		523	52.1	
Screening	Stage 4s	174	63.2							
	Stage 4	154	52.4	0.173						
MNA/no-MNA	Stage 4s	8/206	3.7		0/9	0		0/32	0	
	Stage 4	30/156	16.1	<0.001	18/36	25	0.04	68/186	26.8	<0.001
UFH/FH	Stage 4s	4/95	5.3		0/5	0		4/23	14.8	
	Stage 4	11/78	12.4	0.014	8/16	33.3	0.129	78/97	44.6	0.003
LDH(U/L) (mean level)	Stage 4s	672.4			441.4			675.5		
	Stage 4	1483.8		<0.001	4755.6		<0.001	2316.4		0.019
Therapy										
Observation	Stage 4s	17	6.2		0	0		0	0	
Surgery alone		19	6.9		1	6.2		0	0	
Chemo+surgery		239	86.9		15	93.8		33	100	
Radiation		13	4.7		4	25		13	39.3	
HDT with SCT		4	1.5		0	0		5	15.2	
Observation	Stage 4	0	0		0	0		0	0	
Surgery alone		8	2.7		0	0		0	0	
Chemo+surgery		286	97.3	0.002	73	100	0.017	512	97.9	0.130
Radiation		90	30.6		23	31.5		162	31	
HDT with SCT		19	6.4		20	27.4		142	27.2	
Outcome										
Alive	Stage 4s	258	93.8		15	93.8		17	51.5	
Dead of disease		9	3.3		0	0		12	36.4	
Therapic death		5	1.8		0	0		2	6.1	
Other reason death		1	0.4		0	0		0	0	
Unknown		2	0.7		1	6.2		2	6.1	
Alive	Stage 4	215	73.1		19	26		112	21.4	
Dead of disease		39	13.3		44	60.3		351	67.1	
Therapic death		15	5.1		9	12.3		39	7.5	
Other reason death		3	1		0	0		6	1.1	
Unknown		22	7.5		1	1.4		15	2.9	

Abbreviations: MNA, MYCN amplification; UFH, unfavorable histology; FH, favorable histology; HDT, high dose therapy; SCT, stem cell transplantation.

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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.

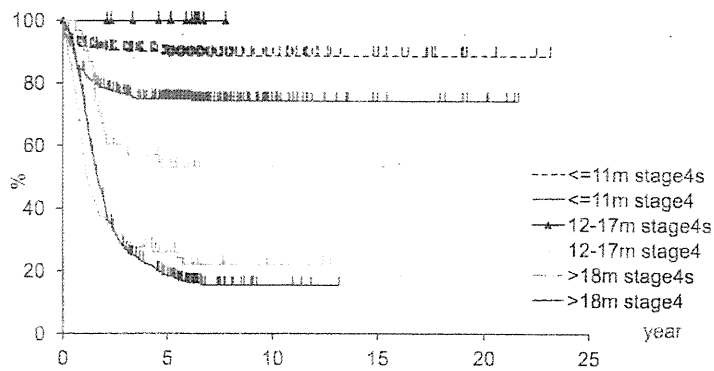
The details of prognosis of each groups were described in Table 1.The prognoses of stage 4s patients were good. Especially, the 5-year overall and event-free survival rates of the cases ⩽11 months of age, 12–17-months of age were excellent (91.2/89.4% and 100/100%, respectively) (Figs. 1 and 2). Comparing 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).

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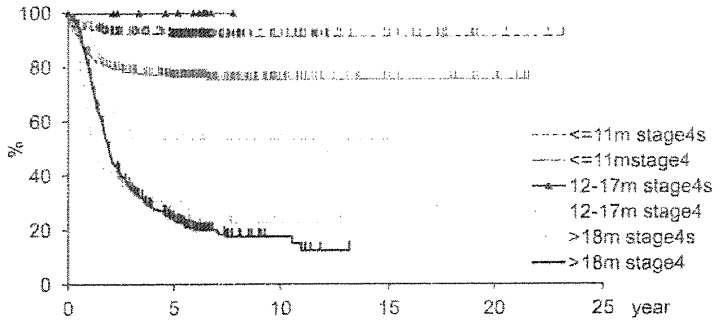

	N	5y-OS	
< = 11m stage 4s	273	91.2%	HRs;1.06
< = 11m stage 4	272	76.3%	95%CI;1.79-4.64, P<0.001
12-17m stage 4s	15	100%	HRs;3.56
12-17m stage 4	72	28.9%	95%CI;2.67-4.63, P=0.007
> = 18m stage 4s	31	53.5%	HRs;2.38
> = 18m stage 4	508	24.4%	95%CI;1.40-4.06, P=0.001

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 ARTICLES

Neuroblastomas with Discordant Genotype-Phenotype Relationships: Report of Four Cases with *MYCN* Amplification and Favorable Histology

Atsuko Nakagawa,[1*] Kentaro Matsuoka,[1] Hajime Okita,[1] Hideto Iwafuchi,[2] Hisanari Hori,[3] and Masaaki Kumagai[4]

[1]Department of Pathology, National Center for Child Health and Development, Tokyo, Japan
[2]Department of Pathology, Aichi Medical University Hospital, Aichi, Japan
[3]Department of Pediatrics, Aichi Medical University Hospital, Aichi, Japan
[4]Department of Hematology, National Center for Child Health and Development, Tokyo, Japan

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ABSTRACT

MYCN amplification prevents cellular differentiation and promotes mitotic and karyorrhectic activities in neuroblastomas. Hence, *MYCN*-amplified tumors typically show an appearance of neuroblastoma of either an undifferentiated or a poorly differentiated subtype with a high mitosis-karyorrhexis index. In addition, they are classified as part of the unfavorable histology group, according to the International Neuroblastoma Pathology Classification. Large cell type and/or presence of prominent nucleoli is also reported to be an additional hallmark of *MYCN* amplification. However, there are few neuroblastomas having *MYCN* amplification and favorable histology. Four cases of *MYCN* amplification and favorable histology were identified in our file of 63 cases of neuroblastoma. The patients (M:F = 3:1) were diagnosed between 6 and 13 months of age, and all had adrenal primary tumors and were treated with high-dose therapy and autologous stem cell rescue. Three patients (stages 1, 3, and 4) are alive and well 7 years, 26 months, and 19 months after diagnosis, respectively. One patient with stage 4 disease died 8 months after diagnosis. Their tumors showed the same histologic feature of neuroblastoma: poorly differentiated subtype with a low mitosis-karyorrhexis index; they were not qualified as large cell type and had no prominent nucleoli. *MYCN* amplification of those tumors was confirmed by fluorescence in situ hybridization in all 4 cases, but MYCN protein expression was not demonstrated by immunohistochemistry (4 cases) and *MYCN* mRNA was not detected by reverse transcriptase polymerase chain reaction (1 case). Those cases showed a discrepant genotype-phenotype that was not simply a laboratory observation but could indicate the concept that that *MYCN* amplification did not automatically equate to a poor prognosis in this group of patients.

Key words: favorable histology, *MYCN* amplification, neuroblastoma

INTRODUCTION

Peripheral neuroblastic tumors (PNTs) (including neuroblastoma, ganglioneuroblastoma, and ganglioneuroma) offer one of the best models for investigating biologically and clinically relevant relationships between their molecular/genetic alterations and morphologic manifestations. Reproducible correlation of *MYCN* gene amplification, an indicator for a poor prognosis, and specific histologic appearance in PNTs has been documented. *MYCN*-amplified tumor has no or limited potential of neuroblastic differentiation and is characterized by markedly increased mitotic and karyorrhectic activities. Therefore *MYCN*-amplified neuroblastoma is usually evaluated as either an undifferentiated or a poorly differentiated subtype with a high mitosis-karyorrhexis index (MKI) and unfavorable histology (UH), according to the International Neuroblastoma Pathology Classification (INPC) [1,2]. It is also reported that there is a significant association between *MYCN* amplification and specific cytologic features, such as large cell type and presence of

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*Corresponding author, e-mail: nakagawa-a@ncchd.go.jp

prominent nucleoli [3–6], also indicating aggressive behavior of PNTs. In contrast, favorable histology (FH) tumors are usually known to have a nonamplified *MYCN* gene.

There are, however, rare PNT cases presenting with a discordant genotype-phenotype relationship. In this report, we describe 4 cases whose tumors had amplified *MYCN* but were classified into the FH group and summarize those cases with the same genotype-phenotype discordance in literature.

MATERIALS AND METHODS

Four cases of neuroblastomas with *MYCN* amplification and FH (*MYCN*-A&FH) were identified among 63 cases of PNTs from the Department of Pathology, Aichi Medical University, and the National Center for Child Health and Development. The patients' clinical information was obtained from the medical records of each institution. The appropriate written informed consent was obtained from the parents. A presentation of the cases follows.

Case 1. This patient presented with an abdominal mass at the age of 6 months. Clinical study with computer tomography demonstrated a right suprarenal tumor. After complete resection of this stage 1 neuroblastoma, the patient received radiation therapy in the tumor bed and chemotherapy (vincristine and cyclophosphamide) for 6 months. Ten months after completion of the chemotherapy, she developed bone recurrence in the right 6th rib. Then she received reinduction/high-dose therapy (cyclophosphamide, vincristine, pirarubicin, cisplatin, etoposide/melphalan, etoposide, and carboplatin) and total body irradiation with autologous peripheral blood stem cell transplantation (PBSCT). The patient is alive and well 91 months after diagnosis.

Case 2. This patient presented with intra-abdominal bleeding due to the rupture of a right adrenal tumor at the age of 9 months. After incomplete resection of stage 3 neuroblastoma, the patient received induction/high-dose chemotherapy (cyclophosphamide, vincristine, pirarubicin, cisplatin/melphalan, etoposide, and carboplatin) with autologous PBSCT and radiation therapy. He has been followed with 13-*cis*-retinoic acid and is now alive and well 26 months after diagnosis.

Case 3. This patient presented with a right adrenal tumor and a nodular lesion of 3 cm in diameter in the right lung (stage 4) at the age of 13 months. After having the diagnosis of adrenal neuroblastoma by biopsy, the patient received induction/high-dose chemotherapy (cyclophosphamide, vincristine, pirarubicin, cisplatin/melphalan, etoposide, and carboplatin) with autologous PBSCT. However, he had tumor progression and died 8 months after diagnosis.

Case 4. This patient presented with an adrenal mass and bone marrow metastasis (stage 4) at the age of 13 months. After having biopsy diagnosis of neuroblastoma from both lesions, the patient was treated with induction/high-dose chemotherapy (cyclophosphamide, vincristine, pirarubicin, cisplatin/melphalan, etoposide, and carboplatin) and autologous PBSCT. He is alive and well 19 months after diagnosis.

Formalin-fixed, paraffin-embedded tumor specimens from the 4 patients were obtained at the time of diagnosis and before chemotherapy/irradiation therapy to determine the *MYCN* status and evaluate the histology/cytology. *MYCN* gene amplification was confirmed by dual-color fluorescence in situ hybridization using a Spectrum green-labeled chromosome 2p telomere probe (TelVysion 2p SpectrumGreen, Vysis, Downers Grove, IL, USA) and a Spectrum orange-labeled locus-specific *MYCN* probe (Vysis LSI N-MYC, Vysis) [7]. Histologic evaluation (determination of Schwannian stromal development, grade of neuroblastic differentiation, and MKI) was performed with hematoxylin and eosin–stained sections, according to the INPC [1,2], and cytologic evaluation (determination of large cell type or not and presence or absence of prominent nucleoli) was performed using the criteria reported by Tornoczky and colleagues [4].

MYCN protein expression was determined immunohistochemically using the same FFPE specimen from those 4 tumors with anti-MYCN protein mouse monoclonal antibody (ab-1, 1:400, oncogene, Cambridge, MA, USA), according to the standard avidin-biotin-peroxydase method. Antigen retrieval procedure was performed by autoclave heating at 121°C in citrate buffer (pH 7.0) for 7 minutes. For positive and negative controls, MYCN protein immunostaining was also performed on *MYCN* amplified and UH (*MYCN*-A&UH, 10 cases) tumors, *MYCN* nonamplified and UH (*MYCN*-nA&UH, 10 cases) tumors, and *MYCN* nonamplified and FH (*MYCN*-nA&FH, 10 cases) tumors. Negative control reaction was also obtained by deleting the primary antibody against MYCN protein.

In 1 case (case 1), snap-frozen tumor material was available for determining *MYCN* expression status at the mRNA level by reverse transcriptase polymerase chain reaction (RT-PCR). Total RNA was extracted from the tumor using RNeasy Mini kit (Qiagen, Tokyo, Japan) and treated with RNase free DNase I (Qiagen) according to manufacturer's instructions. The oligonucleotide primers for the human *MYCN* gene [sense: (5′) AGT TCC TTC CAC CCT CTC CT; antisense: (5′) CAC CCA GCA ACC CCC TAA AC], specific for a 151–base pair intronic sequence (intron 2), and the PCR conditions were described previously by Gilbert and colleagues [8].

RESULTS

Clinicopathologic characteristics of the 4 cases are summarized in Table 1. As shown in Figure 1, *MYCN* amplification in these tumors was confirmed by fluorescence in situ hybridization. It was noted that all tumors had the same histologic evaluation of neuroblastoma (Schwannian stroma poor): poorly differentiated subtype with a low MKI, according to the INPC (Fig. 2). No tumors had a cytologic appearance characteristic for large