Mass Screening for Neuroblastoma in Japan: Lessons Learned and Future Directions

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Background/Purpose: Since 1985, a nationwide mass screening program (MS) for neuroblastoma has been conducted for 6-month-old infants throughout Japan, resulting in the detection of more than 1,900 cases of neuroblastoma. The outcome of these patients has been excellent: more than 97% of them are alive. Yet, several reports suggest that the number of advanced-stage neuroblastoma patients over 1 year of age has not changed substantially. The current report focuses on the 15-year experience with MS of the Kyushu Pediatric Oncology Study Group.

Methods: The clinical and biological features of neuroblastomas detected (n = 320) and not detected by MS (n = 245) were compared. Regional and national statistics for neuroblastoma before and after 1985 were analyzed using standard epidemiologic measures for the occurrence of disease.

Results: The majority of the MS-positive cases were biologically favorable and had an excellent outcome. In contrast, the majority of non-MS patients in whom neuroblastoma later developed had advanced-stage, unfavorable-prognosis tumors. The overall mortality rate of neuroblastoma in the Kyushu area was not improved by MS.

Conclusions: The optimal time for screening is the point at which neuroblastomas regressing spontaneously can no longer be detected, but more aggressive disease can be found. A birth cohort study could determine the optimal timing for a second screening. Identification of other new prognostic factors may be required.

INDEX WORDS: Neuroblastoma, mass screening, biology, epidemiologic study.

Distinguished Guests, members, ladies and gentlemen, I am delighted and honored to be invited to deliver the Professor Stephen Gans visiting guest lecture and I thank you most sincerely for this opportunity. Professor Gans was a great pediatric surgeon, and of course his accomplishments far outshine mine. I met him several times with Professor Suruga. In November 1992, I had lunch with Professor and Mrs Gans, and Professor Suruga when Professor Gans was invited as a guest speaker at our meeting in Tokyo. At that time, Professor Gans kindly invited me to serve as an editorial consultant for the Journal of Pediatric Surgery. I never expected such an honor. Looking back, that was his last visit to Japan. With this lecture I would like to pay tribute to the memory of Professor Stephen Gans.

Since 1985, a nationwide mass screening (MS) program for neuroblastoma has been conducted for 6-month-old infants throughout Japan.1,2 Today, more than 1,900 Japanese children have had neuroblastoma diagnosed through MS at 6 months of age. The outcome of these patients is excellent, and more than 97% of the patients are alive.3 However, several reports have suggested that the number of advanced-stage neuroblastoma patients over 1 year of age has not changed substantially.4,5 The current study focuses on the 15-year experience from the pediatric oncology study group of the Kyushu area, with a total population of 15 million, and a population less than 15 years old of 3 million people.

MATERIALS AND METHODS

The details of the MS procedure in Japan have been published elsewhere.6 Briefly, all 3-month-old infants underwent a physical examination at the local health center under the Child Health Survey Program. The mother was given a screening kit that contained filter paper, a postage-paid return envelope, and a prospectus for measuring the urinary vanilmandelic acid (VMA), homovanillic acid (HVA), and creatinine. A specimen of urine was collected on the filter paper, dried, and sent to the examination center of each prefecture when the infant

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was 6 months old. Food or drinks containing vanilla were forbidden during the 24-hour period preceding the test. A repeat examination was performed in the case of a positive urine test. If both the first and second tests were positive, the infant was referred to a regional hospital for further investigations including a physical examination, chest and abdominal x-rays and computed tomography (CT), abdominal sonogram, tumor markers, and biological studies. If a mass was detected, then treatment was given without delay. However, if no mass was detected, but the VMA or HVA levels remained high, the infants underwent further examinations; follow-up was continued until the VMA and HVA levels decreased to the normal level.

In the Kyushu area, the presence of urinary VMA and HVA was measured by high-performance liquid chromatography. Biological features studied included MYCN gene amplification, DNA ploidy, Shimada histology, serum neuron-specific enolase (NSE), and serum ferritin. The log-rank test based on the Kaplan-Meier procedure was used to estimate survival rate, and the Student’s t test was used for statistical analysis. This study was approved by Institutional Review Board of Kyushu University.

RESULTS

A total of 565 newly diagnosed cases of neuroblastoma were registered by the Committee for Pediatric Solid Malignant Tumors in the Kyushu Area between 1985 and 2000. Three hundred twenty of these were detected by MS. A total of 245 cases were non-MS cases. Ninety-seven of the 245 non-MS cases occurred in patients who were negative by MS at 6 months, but later demonstrated neuroblastoma. A total of 148 of 245 cases were detected clinically without receiving the MS test. This group includes the infants less than 6 months old in whom neuroblastoma developed before the age of screening, infants whose parents did not submit the sample for analysis, and infants over 6 months old at the start of MS.

Patients With Neuroblastoma Detected by MS

The clinical features of 320 MS cases are presented in Table 1. These cases were classified according to the clinical staging of the Children’s Cancer Study group.6 There were 255 stage I, stage II, and stage IVS; 64 stage III and stage IV; and one with stage unknown. The primary tumor site was adrenal in 145 cases and extra-adrenal in 175 cases. All patients were treated based solely on institutional choice. A complete resection of the tumor was performed in 266 of the 320 patients, an incomplete resection was performed in 43 patients, and no resection was done in 11 patients. No significant difference in survival rate between the complete and an incomplete resections was observed. One hundred ninety-six of the 320 cases received the 2-drug chemotherapy regimen (cyclophosphamide and vincristine), 16 received the aggressive chemotherapy regimen (a combination of cyclophosphamide, vincristine, cisplatin, etoposide, and tetrahydroxypyranyl Adriamycin),7 and 88 had no chemotherapy. The information concerning chemotherapy in the rest of 20 is unknown. Of the 237 patients with stage I and II disease, 142 cases received 2-drug chemotherapy, whereas 82 cases had no chemotherapy. In these patients with stage I and II, no significant difference in survival rate was observed in the children who had received chemotherapy versus those who had not.

Survival rate in cases detected by MS was 315 of 320 (98.4%). Five patients of all 320 MS cases died, but only one of them died of the disease because of brain metastasis. One patient with stage III died of cardiac complications caused by the side effects of chemotherapy during therapy in complete response (CR; 131 postoperative days) and another stage III patient died of operative side effects (1 postoperative day). One stage II patient died of viral nephritis after completion of therapy in CR (1,626 postoperative days) and another stage II patient died of gastrointestinal bleeding after completion of therapy in CR (270 postoperative days).

The biological features of 320 MS cases are presented in Table 1. Some cases were not examined for one or more of the biological features. The total numbers examined were as follows: MYCN gene, 285; DNA ploidy,
131; Shimada histology, 228; NSE, 270; and ferritin, 188. None of the 285 examined cases had more than 10 copies of MYCN gene, and 7 had 2 to 9 copies (2, 2.7, 3, 4, 5, 5.7, 6 copies, respectively). One hundred five of the 131 examined tumors had a favorable ploidy pattern (aneuploidy), whereas 26 had an unfavorable ploidy pattern (diploid or tetraploid). With regard to Shimada’s histologic classification, 220 of the 228 examined tumors were considered to have a favorable histology; the other 8 had an unfavorable histology. Of the 270 cases with available serum NSE levels, 6 cases showed elevations (>100 ng/mL). Of the 188 cases with available serum ferritin levels, 5 cases exhibited elevations (>150 ng/mL). A total of 47 cases (14.7%) presented with one or more biologically unfavorable factors.

**Patients With Neuroblastoma After an Initial Negative Screen (Non-MS Cases)**

The clinical and biological features of 97 non-MS cases who later had neuroblastoma are presented in Table 2. They were all negative at 6-month MS; neuroblastoma was diagnosed at a mean age of 3.1 ± 2.1 years. Of 97 non-MS cases, 81 cases (83.5%) were stage III or stage IV. Fifty-eight (59.8%) of 97 non-MS patients had elevated urinary VMA or HVA excretion. 39 of the 71 examined cases had elevated urinary VMA and HVA excretion, 4 showed an increase in the VMA excretion only, and 15 had an increase in HVA excretion only. Some cases were not examined for one or more of the biological features. The total numbers examined were as follows: MYCN gene, 73; DNA ploidy, 26; Shimada histology, 22. Twenty-six cases of the 73 examined cases had more than 10 copies of MYCN gene. Eighteen tumors of the examined 26 had an unfavorable ploidy pattern (diploid or tetraploid). With regard to Shimada’s histologic classification, 15 of the 22 examined tumors were considered to have an unfavorable histology. Forty-five (46.4%) of 97 non-MS patients died.

![Fig 1. Survival curve of MS cases, non-MS cases, and unscreened cases.](image)

**Table 2. Clinical and Biological Features of 97 Non-MS Cases**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>Stage I, II, IVS</td>
<td>16</td>
</tr>
<tr>
<td>Stage III, IV</td>
<td>81</td>
</tr>
<tr>
<td>Urinary VMA and HVA (n = 81)</td>
<td></td>
</tr>
<tr>
<td>VMA and HVA</td>
<td>39</td>
</tr>
<tr>
<td>VMA only</td>
<td>4</td>
</tr>
<tr>
<td>HVA only</td>
<td>15</td>
</tr>
<tr>
<td>Not elevated</td>
<td>23</td>
</tr>
<tr>
<td>MYCN amplification (n = 73)</td>
<td></td>
</tr>
<tr>
<td>&lt;10 copies</td>
<td>47</td>
</tr>
<tr>
<td>≥10 copies</td>
<td>26</td>
</tr>
<tr>
<td>DNA ploidy (n = 26)</td>
<td></td>
</tr>
<tr>
<td>Aneuploid</td>
<td>8</td>
</tr>
<tr>
<td>Diploid or tetruploid</td>
<td>18</td>
</tr>
<tr>
<td>Shimada histology (n = 22)</td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>7</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>15</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>52</td>
</tr>
<tr>
<td>Death</td>
<td>45</td>
</tr>
</tbody>
</table>

Of 320 MS cases, 6 patients had recurrence with local or metastatic disease (Table 3). Three of the 6 relapsed patients underwent a complete resection of the primary tumor, whereas 3 cases had an incomplete resection of the tumor. Regarding the initial chemotherapy, 3 cases received 2-drug chemotherapy (cyclophosphamide and vincristine), 2 cases received no chemotherapy, and 1 case had high-dose multidrug chemotherapy (cyclophosphamide, etoposide, tetrahydro-pyranyl Adriamycin, cisplatin). The time from initial therapy to recurrence ranged from 3 months to 48 months. There were 2 local recurrences and 4 metastatic recurrences. Regarding biological prognostic factors, 4 of 6 relapsed cases had one or more unfavorable factors, whereas 2 cases had no unfavorable factors. Only one case (case 5) had MYCN amplification (5.7 copies). As for the treatment for recurrence, surgical intervention for local recurrences was performed in 2 patients (case 1 and 2), high-dose multidrug chemotherapy for 5 patients, and a peripheral blood stem cell transplantation was performed for 3 cases.
Regarding the outcome after relapse, 4 patients are CR, 1 patient has a stable residual tumor, and 1 patient died of disease (brain metastasis) with MYCN amplification.

Epidemiologic Study Evaluating the Effectiveness of MS at 6 Months of Age

As shown in Fig 2, the incidence of neuroblastoma has increased in Japan since the institution of MS in 1985. However, the mortality rate from neuroblastoma has not changed significantly. Consistent with these observations, the case fatality rate has decreased since 1985 (Fig 3). The retrospective cohort study has shown the following: in children less than 5 years of age, born from 1988 to 1992, the cumulative incidence of neuroblastoma was 82 in 484,599 for screened children, and 11 in 92,966 for unscreened children, respectively. Fourteen of the 82 screened patients were negative at 6 months of age (non-MS cases).\(^{10}\) The cumulative incidence rates (per 100,000) at more than 6 months and less than 1 year of age were 14.0 in the screened infants, and 2.2 in the unscreened infants, respectively \((P < .01)\). However, no significant difference was found in the crude cumulative incidence at more than 1 year and less than 5 years of age between the screened and the unscreened infants \((2.9 \text{ v. } 2.2, \text{ respectively; } P = .959)\). Furthermore, no significant difference was observed in the cumulative mortality rates at less than 5 years of age between the screened children \((1.4)\) and the unscreened children \((0.0; P = .556)\).

Patients With Neuroblastoma Detected by MS, But Managed by Observation Only

Recent reports describe the clinical course of MS cases managed with nontreatment and observation (no surgical intervention and no chemotherapy).\(^{11}\) The Committee for the Japanese Association of Pediatric Oncology analyzed 82 MS cases at 17 institutions managed by nontreatment and observation in 1998.\(^{12}\) The criteria for this strategy was dependent on each institute, but roughly the following

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Stage</th>
<th>Operation of Primary Tumor and (Initial Chemotherapy)</th>
<th>Time From Initial Therapy to Relapse (Pattern of Relapse)</th>
<th>Unfavorable Biological Features</th>
<th>Follow-Up Period After Relapse</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>Complete resection (−) (multiple, bone and local site)</td>
<td>3 mo</td>
<td>None</td>
<td>96 mo</td>
<td>CR</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>Complete resection (−) (bone and bone marrow)</td>
<td>21 mo</td>
<td>Diploid, low expression of TrkA</td>
<td>51 mo</td>
<td>CR</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>Partial resection (CPA, VCR) (bone and bone marrow)</td>
<td>18 mo</td>
<td>Tetraploid</td>
<td>80 mo</td>
<td>NC</td>
</tr>
<tr>
<td>4</td>
<td>III</td>
<td>Complete resection (CPA, VCR) (increase of residual tumor)</td>
<td>36 mo</td>
<td>Low expression of TrkA</td>
<td>55 mo</td>
<td>CR</td>
</tr>
<tr>
<td>5</td>
<td>III</td>
<td>Biopsy (New A1) (brain metastasis)</td>
<td>10 mo</td>
<td>MYCN: 5.7 copies</td>
<td>1 mo</td>
<td>DOD</td>
</tr>
<tr>
<td>6</td>
<td>III</td>
<td>Partial resection (CPA, VCR) (local recurrence)</td>
<td>48 mo</td>
<td>Shimada: unfavorable NSE ≥100 ng/mL</td>
<td>27 mo</td>
<td>CR</td>
</tr>
</tbody>
</table>

Abbreviations: CPA, cyclophosphamide; VCR, vincristine, New A1, cyclophosphamide, etoposide, tetrahydro-pyranl Adriamycin, cisplatin; CR, complete response; NC, no change; DOD, died of disease.
criteria: Evans Stage I, II, or IVS; tumor less than 5 cm in diameter; no invasion to the intraspinal canal or to the great vessels; urinary VMA and HVA levels less than 50 μg/mg creatinine; and informed consent obtained from parents. Twenty-two of the 82 cases subsequently underwent surgical intervention between 11 to 57 months (average, 17 months) because of an increased tumor size, with or without an increase in urinary VMA and HVA levels (n = 15) or at the parents’ request (n = 7). Histologic findings in the 22 cases showed neuroblastoma in 10, ganglioneuroblastoma in 11, and ganglioneuroma in 1, respectively. Of the 60 cases initially observed without surgical intervention, tumors disappeared in only 17 cases, whereas tumor persisted in 43. Follow-up study for these 82 cases in 2000 found that 24 tumors were completely removed. In 24 cases (among 58 patients observed without surgical intervention), the tumor disappeared, confirmed by CT; the tumor persisted in 34 cases. All 82 patients in the cohort are still alive and have the normal range of urinary VMA and HVA.

**DISCUSSION**

These findings suggest that: (1) the majority of the patients detected by MS had a favorable prognosis and (2) MS at 6 months of age did not reduce the incidence and mortality from neuroblastoma. The majority of neuroblastomas detected by MS in Kyushu at 6 months of age had biologically favorable factors, and the outcome of these patients has been excellent. However, approximately 14.7% of the cases had one or more unfavorable factors and experienced a higher relative risk of relapse. Our study suggests that biologically unfavorable factors were associated with a high risk of recurrence in MS cases, although some relapsed cases were observed that had no unfavorable factors. Hence, long-term follow-up for MS cases is important. It also may be necessary to better define the significance of established biological factors and to identify new prognostic factors.

Epidemiologic studies, including a retrospective cohort study, showed that MS at 6 months of age in Japan has not substantially improved the prognosis of patients with unfavorable neuroblastoma identified over 1 year of age. The patients who were initially MS negative but who later had neuroblastoma, were likely to present with advanced-stage and poor-prognosis disease.

The report of nontreatment and observation of MS cases investigated by the Committee for the Japanese Association of Pediatric Oncology might suggest that a subset of tumors detected by MS at 6 months of age have the capacity to either spontaneously regress or mature. However, the clinical course of the cases that were observed without treatment are not necessarily representative of the natural course of neuroblastoma detected by MS, because the described cases may have been atypical: they included few patients with stage I or II disease, were associated with relatively low levels of urinary VMA and HVA, and did not invade surrounding organs and vessels. Therefore, these findings do not support management by nontreatment and observation. It is possible that MS may allow the determination of biologic factors in neuroblastomas detected at 6 months of age, which then may potentially predict whether a neuroblastoma will spontaneously regress or aggressively grow.

Hypothetically, the optimal time for screening is the point at which the cases regressing spontaneously would not be detected, but aggressive tumors would be found at an early stage. We suggest that this time point may be defined by examining the following data: (1) the accumulation of the follow-up data of cases detected by MS but not treated, which may help to determine the time course of spontaneous regression and (2) a prospective cohort study, analyzed for biological factors at later ages. The latter study may help to clarify whether a second screening can detect unfavorable neuroblastomas at an early stage.

**ACKNOWLEDGMENTS**

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