Review

Screening for Neuroblastoma: Progress and Pitfalls[1]

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The primary goal of cancer screening is to identify tumors in their earlier and, presumably, more curable stages. Mass screening has been recommended for the early detection of several adult malignancies, including cancers of the colon, breast, cervix, and prostate. In the past two decades, population screening has been implemented in several countries for the early detection of neuroblastoma, one of the most common pediatric cancers, and a great deal has been learned about the strengths and limitations of screening. Here, we review and discuss the neuroblastoma screening experience.

Epidemiology of Neuroblastoma

Neuroblastoma is a malignancy of early childhood, affecting ~1 in 7000 children (1). It is the most common extracranial solid tumor that occurs in children, with an overall incidence rate in the United States of ~9.7 per million children under the age of 15 years (2, 3). There is a modest difference in incidence rates by sex (boy:girl rate ratio = 1.2). The highest incidence rate, 55.2 per 1 million, occurs in children in the first year of life (3). Neuroblastoma is extremely rare in children older than 5 years of age. White children have a slightly higher incidence (25%) than black children (3). Prior to screening, highest rates for neuroblastoma were reported in the United States (whites), Israel (Jews), New Zealand (Maori), and France (range, 11–14 per million); intermediate rates occurred in Japan, the United States (blacks), and the United Kingdom (range, 7–9 per million); and lowest rates were reported in India and China (range, 3–5 per million; Ref. 4). There has been a notable change in incidence rates in countries that have implemented screening, which will be discussed in more detail below.

A small percentage of patients with neuroblastoma exhibit a genetic predisposition to the disease, with an autosomal dominant pattern of inheritance (2, 5). In these familial cases, the median age at onset is 9 months, in contrast to a median age of 22 months in the general population (6). For the remaining majority of children with neuroblastoma, however, the etiology is largely unknown. Due to the early age of onset, several epidemiological studies have focused on preconceptional and prenatal events. There has been a suggestion that maternal exposure to certain medications and hormones during pregnancy is associated with an increased risk of neuroblastoma (7, 8). A recent study reported a statistically significant 2-fold increased risk of neuroblastoma associated with increasing birth weight (9). Overall, epidemiological investigations of neuroblastoma to date have not been very informative.

Clinical Behavior of Neuroblastoma

The most important clinical factors determining outcome for neuroblastoma are the age of the child at presentation and the stage of the disease (10). For all stages of disease beyond localized tumors, infants (diagnosed under 1 year of age) have significantly better disease-free survival than older children with equivalent stages of disease. Advanced stage neuroblastomas, generally presenting in children over the age of 1 year, have an aggressive clinical course with poor survival despite modern intensive therapies. In contrast, early stage neuroblastomas presenting in younger children have an excellent prognosis with survival rates >90%. A particular pattern of disease termed stage 4S, with a small primary tumor and metastases to liver and/or skin only occurring in infants has been particularly associated with spontaneous regression in the absence of treatment (11–13).

A number of different staging criteria have been developed for neuroblastoma. The use of different staging systems in early studies made comparison of results of clinical trials difficult. An international working group met in 1986 to establish criteria for a common neuroblastoma staging system based on clinical and surgery copathological findings (14). The system, known as the INSS,[3] is now widely used (Table 1). The staging system was revised in 1993 and retrospective analyses of patients treated by the Pediatric Oncology Group and Children's Cancer Group have confirmed that these criteria identify prognostic subsets of patients with neuroblastoma (15–17). Of note, the distribution of patients according to stage differs depending on age at diagnosis. In patients diagnosed at <1 year of age, the majority have resectable or nonmetastatic disease, whereas almost 80% of patients diagnosed over the age of 1 year will have stage 3 or 4 disease and a poor prognosis.

Biological Prognostic Factors

Biological characteristics of the primary neuroblastoma tumor have also been shown to have a prognostic importance and have important implications for screening programs. The early observation of the presence of double minute chromosomes and homogeneously staining regions in the chromosomes of neuroblastoma tumors led to the identification of *MYCN* gene amplification in a proportion of neuroblastomas (18, 19). *MYCN* is a proto-oncogene and appears to contribute to the more aggressive behavior of some neuroblastomas (20). The frequency of amplification of *MYCN* is low in patients with less advanced stages of disease (~5% of low-stage tumors and

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[3] The abbreviations used are: INSS, International Neuroblastoma Staging System; VMA, vanillylmandelic acid; HVA, homovanillic acid; HPLC, high-pressure liquid chromatography; GC-MS, gas chromatography-mass spectrometry; EIA, enzyme immunoassay; QNSP, Quebec Neuroblastoma Screening Project.

Table 1 INSS[a]

Stage	Characteristics
1	Localized tumor confined to the area of origin; completed gross excision, with or without microscopic residual disease; identifiable ipsilateral and contralateral lymph nodes negative microscopically.
2A	Unilateral tumor with incomplete gross excision; identifiable ipsilateral and contralateral lymph nodes negative microscopically.
2B	Unilateral tumor with complete or incomplete gross excision; with positive ipsilateral regional lymph nodes; identifiable contralateral lymph nodes negative microscopically.
3	Tumor infiltrating across the midline with or without regional lymph node involvement; unilateral tumor with contralateral regional lymph node involvement; or midline tumor with bilateral lymph node involvement.
4	Dissemination of tumor to distant lymph nodes, bone, bone marrow, liver, or other organs (except as defined in stage 4S).
4S	Localized primary tumor as defined for stage 1 or 2 with dissemination limited to liver, skin, or bone marrow.

[a] Taken from Refs. 2 and 15. (Permission granted to reproduce table from Dr. Brodeur and W. B. Saunders Company, Orlando, FL).

Table 2 Prognostic characteristics of neuroblastoma[a]

Characteristic	Most favorable	Least favorable
Age (yr)	<1	1–5
INSS stage	1, 2, or 4S	4
MYCN gene	Not amplified	Amplified
Ploidy	Hyperdiploid	Diploid
Histology (Shimada classification)	Favorable	Unfavorable
1p loss of heterozygosity	Absent	Present
TRK-A expression	High	Low or absent
Survival	Excellent (>95%)	Poor (<20%)

[a] Adapted from Refs. 18–40.

~40% of high-stage tumors). A number of excellent clinical studies have shown that *MYCN* amplification is associated with an inferior outcome in patients with advanced stage disease treated with conventional chemotherapy (18, 20–23). While the majority of patients with *MYCN*-amplified tumors will have disseminated disease that will behave in an aggressive manner, the prognostic importance of *MYCN* gene amplification in localized neuroblastoma or patients with INSS stage 4S disease is controversial (22, 24).

The total DNA content of tumor cells, usually measured by flow cytometry, may show an increased DNA content (DNA index > 1), designated hyperdiploid, or a normal DNA content (DNA index = 1), termed diploid. Infants with hyperdiploid tumors have been shown to be more likely to have low stages of disease at diagnosis and a more favorable response to chemotherapy regardless of stage (23, 25–27). In older patients, ploidy is a less important determinant of outcome.

Variable expression of TRK-A (the receptor for nerve growth factor) has also been reported in neuroblastoma (26–33). High expression of TRK-A is associated with other favorable prognostic features (normal MYCN copy number, low stage, and young age).

Deletion or allelic loss (loss of heterozygosity) of the short arm of chromosome 1 is correlated with inferior survival in patients with neuroblastoma, although it is not yet clear whether this finding represents an independent risk factor because most cases in which 1p deletions are noted also have MYCN amplification (33–36). The 1p36 region has been a target of interest and investigation for molecular biologists and a gene termed *p73* has recently been identified at this locus that appears to be a good candidate for a "neuroblastoma tumor suppressor gene" (37, 38). p73 has homology to the tumor suppressor gene *p53*, activates the transcription of p53-responsive genes, and inhibits growth in a p53-like manner by inducing apoptosis.

Histopathologically, neuroblastoma may present a wide spectrum of maturation and differentiation. Like staging, the histopathological criteria for tumor classification have varied. The most widely used system was developed by Shimada and colleagues (39) and subsequently revised by Joshi *et al.* (40), and it classifies tumors as favorable or unfavorable characteristics in terms of likely clinical outcome.

Table 2 summarizes the clinical and biological characteristics of the most favorable and unfavorable neuroblastomas. In limited-stage neuroblastoma with favorable biological characteristics, outcome is excellent, with survival of >95% and spontaneous regression of disease in the absence of therapy occurring in some cases. In patients with the least favorable disease, age is greater, disease is disseminated, and survival is poor despite intensive chemotherapy, illustrating the broad spectrum of biological behaviors of these diseases. It remains uncertain whether a proportion of patients with favorable disease will progress to more aggressive disease or whether these represent two completely different diseases with no movement between the two categories. The close linkage of age with stage supports the belief that good prognosis tumors progress over time to bad prognosis tumors, which was important in the establishment of neuroblastoma screening programs. However, recent data from screening studies (see below) and from biological studies tend not to support movement from good to poor prognosis disease (41).

Screening for Neuroblastoma

Some of the general criteria for implementation of a cancer screening program include: (*a*) the cancer is an important public health problem; (*b*) effective treatment is available, and the treatment is more effective if given earlier rather than later; (*c*) there is a suitable screening test available (*i.e.*, it is reproducible, valid, acceptable, and cost-effective); (*d*) there is a reasonably long and/or detectable presymptomatic period; and (*e*) the natural history of progression of the malignancy is understood (42, 43). These criteria are addressed below with respect to neuroblastoma.

Public Health Problem. The majority of older children diagnosed with advanced-stage neuroblastoma will die of the disease. Although neuroblastoma is relatively rare compared to adult tumors, the potential years of life lost are substantial. Moreover, neuroblastoma is nearly twice as common as phenylketonuria and 10 times more common than galactosemia, two pediatric diseases that are routinely screened for in the United States (44).

Effective Treatment Is Available, and Treatment Is More Effective if Given Earlier Rather than Later. Children with advanced-stage disease do not fare well. If neuroblastoma progresses from a more favorable biology to a less favorable biology, then it is appropriate to initiate screening at the earliest possible age.

Suitable Screening Tests Are Available. The majority of neuroblastomas secrete catecholamines, and elevated levels of the metabolites VMA and HVA can be detected in urine, allowing for easy and relatively inexpensive screening assays (Table 3). Urinary HVA and VMA have been assayed by a number of different techniques including the spot test, TLC,

Table 3 Screening methods used to detect neuroblastoma[a]

Method	Where used	Comment
VMA spot test	Early experimental Japanese studies	Qualitative test based on a color change on filter paper; not very sensitive or specific (missed ~30% of cases and had false positives of ~5%); had dietary interference; no longer used
TLC	Quebec study for primary screen	A rapid, reproducible, and relatively inexpensive test; qualitative and, thus, not very sensitive; need additional method of analysis for all positive tests (such as GC-MS)
GC-MS	Quebec (TLC positives)	Quantitative, very sensitive, and specific; labor-intensive and expensive to conduct; however, 99.99% specificity in combination with TLC; 44% predictive value positive
	England (primary screen)	
HPLC	Japan (primary screen)	Sensitive and specific; complex and relatively expensive in comparison to TLC but lower incidence of false positives
EIA	Austria (primary screen)	Minimal equipment is required so relatively inexpensive; however, high number of false positives requires a confirmatory method such as HPLC
	Texas (primary screen)	
	Japan	

[a] From Refs. 45–52.

HPLC, GC-MS, and EIA (45–52). The European Neuroblastoma Study Group has reported that 87% of stage III and IV tumors excrete elevated levels of HVA and/or VMA, compared to 64% of stage I and II tumors (53). Tumors that do not excrete VMA/HVA (30–40% of low-stage tumors and ~20% of high-stage tumors) will not be detected by current screening strategies.

There Is a Reasonably Long or Detectable Presymptomatic Stage, and the Natural History of the Disease Should Be Understood. At the time of screening implementation, it was presumed that early-stage good prognosis tumors will, if untreated, evolve into aggressive tumors with high mortality so that early detection would provide a substantial benefit to patients.

Summary of Screening Studies

Japan

Japan has been the pioneer in neuroblastoma screening. Experimental screening studies were initiated on a small scale in health centers in Kyoto in the early to mid-1970s, followed by further testing in other health centers and districts in Japan (reviewed in Ref. 54). In these experimental studies, infants were screened at 6 months of age due to practicalities in the timing of health care visits. Parents collected a urine sample on filter paper from their infant and sent it to the investigators. These initial screening trials used the VMA spot method, but upon recognizing the limitations of this method, all screening programs switched to the more sophisticated HPLC method (see Table 3). Subsequently, results of these studies were published regarding the benefits of screening at 6 months of age (50, 55, 56). Overall, the children identified by these experimental screening programs were in the early stages of the disease and experienced a superb outcome. Assuming that these were cases that would have progressed to advanced-stage disease, the experimental trials were considered a success. Japan implemented a nationwide screening program in 1985.

By the late 1980s, it was recognized by investigators both in and outside Japan that these early observations were methodologically flawed (57–62). Goodman (54), in an excellent review and analysis, applied rigorous epidemiological methods to the screening data from Japan and suggested extreme caution in interpreting the Japanese results without age-specific population-based incidence and mortality rates. Others reached similar conclusions (63–67). Overall, the consensus was that the neuroblastoma screening program in Japan was largely diagnosing tumors that would have never manifested clinically (63). In fact, screening at 6 months resulted in more than a doubling of the cumulative incidence of the disease before the age of 5 (1 of 3515 after screening *versus* 1 of 8400 before screening; Ref. 65).

In response to these observations, investigators from Japan have suggested discontinuation of mass screening of infants at 6 months of age (68). Recognizing that neuroblastoma is likely to be at least two biologically distinct diseases, studies are currently being conducted to explore the potential benefit of screening only at 12 and 18 months of age.

North America

QNSP. The QNSP was initiated to determine whether mass screening could reduce overall mortality of neuroblastoma in a large population of infants (69, 70). A unique aspect to this study was the inclusion of two concurrent nonscreened population-based control groups, including the state of Minnesota and the province of Ontario. The QNSP project was implemented in the province of Quebec in 1989 and offered parents the opportunity to have their infant's urine tested at 3 weeks and 6 months of age. (The 3-week screen was performed on a urine sample that is routinely collected to screen for various metabolic diseases.) There were overall compliances of 91% on the first screen and 74% on the second (71). As of July 1995, with a follow-up of the cohort for 15–75 months, there were 118 cases of neuroblastoma diagnosed, including 43 detected preclinically by screening. On the basis of Surveillance, Epidemiology, and End Results program data, only 54.5 cases would have been expected during this period. When age-specific groups were examined, there was a substantial increase in the incidence of cases diagnosed in the first year of life, with no reduction in incidence for subsequent years (71). When the analysis was limited to advanced-stage disease diagnosed over the age of 1, no reduction in incidence was observed. The authors concluded that screening only increases the incidence of neuroblastoma in infants with no reduction in the incidence of advanced-stage disease. This finding, similar to the Japanese experience, suggests that early-stage neuroblastoma with good biological features does not generally progress to aggressive disease with high mortality. In support of this, Woods *et al.* (72) recently reported that examination of preliminary standardized mortality analyses on the screened cohort suggests no reduction in overall mortality from the disease.

Texas Outreach Program for Neuroblastoma Screening. This program was initiated between February 1991 and June 1994 to determine the feasibility of neuroblastoma screening in a United States population of infants ages 5–10 months (73). Screening kits that contained instructions and filter paper for obtaining urine were prepared for parents. Kits were distributed to parents in private physician offices, in public health clinics, and by mail. Of 291,158 screening kits distributed, only 14,046 were returned, for an overall compliance of 4.8% (the highest compliance was in the Houston-area Women, Infants, and Children clinics: 53%). ELISAs were performed on the returned screened specimens, and neuroblastoma was ultimately diagnosed in two screened infants. The authors recognized that they could make no recommendation regarding the value of neuroblastoma screening (73). Their data are interesting, however, with respect to compliance rates for a childhood screening program in a diverse United States population.

France

A pilot study of HPLC screening at 4 months of age was initiated in the Rhone French district between 1990 and 1994 (74). Overall compliance was 81%. Twelve cases were detected by screening; only 2 of 12 demonstrated unfavorable biological characteristics. In the second screening program in France, initiated in 1995, infants are being screened at 12 months of age. Thus far, compliance has been ~60%, and four cases have been detected by screening, all with favorable biology (75).

Germany

In a pilot screening study initiated in 1991 in Hamburg and Stuttgart (using HPLC), 11 cases of neuroblastoma were detected by screening 99,490 infants (mean age at diagnosis was 9.7 months; Refs. 76 and 77). Only 2 of 11 of these tumors demonstrated unfavorable biological characteristics. Mass screening was initiated in 1995 at 10–14 months of age (77, 78). It is expected that over 1.2 million infants will participate and that approximately the same number will be followed as a control group (78). This study should have sufficient population-based data (*e.g.*, incidence and mortality) to determine the efficacy of screening at this age.

Austria

Screening was initiated in 1990 in infants ages 7–12 months (79). Of a total of 200,488 infants screened using EIA with HPLC as a backup, 24 neuroblastomas have been detected. Of these cases, 4 of 24 had MYCN amplification (80). Although the authors observed a slight increase in neuroblastoma incidence, there was a concurrent decrease in incidence of stage 4 disease diagnosed in children older than 1 year of age (5.2 *versus* 4.0 cases). These results are extremely preliminary and need to be substantiated with long-term follow-up incidence and mortality data.

England

Four health districts in Northern England have been targeted for infant screening at 6 months of age (81). Urine was collected on filter paper and analyzed by GC-MS. A total of 20,829 infants were screened between 1987 and 1990, which comprised 92% of the target population. Two infants with neuroblastoma were detected by screening; both are alive and well (80). It is important to note, however, that efficacy could not be evaluated without a comparable control population.

Overall Evaluation of the Neuroblastoma Screening Studies

Four basic epidemiological principles must be addressed in determining the overall effectiveness of these screening programs: selection bias, lead time bias, length-time bias, and detection bias (54, 82).

Selection bias is the concern that individuals who participate in a screening program are somehow "different" (and, thus, have a better prognosis) than individuals who do not. In the case of neuroblastoma, it is unlikely that this would be a problem because nothing is known about the etiology of the disease that might lead to self-selection of cases.

Lead time is the period between screening diagnosis and the clinical diagnosis. If early detection had no effect on the course of the disease and everyone with the disease eventually died, one could incorrectly assume that screening prolonged survival. For neuroblastoma, this is also an unlikely problem. One method to evaluate lead time bias is to compare age-specific incidence or (ideally) mortality rates between a screened population and an unscreened population. In the Quebec study, there was no overall reduction in incidence in children diagnosed over the age of 1 year post-screening, compared to similar unscreened populations in Minnesota and Ontario (71).

Length-time bias occurs when tumors detected by screening have a longer preclinical stage and are slower growing than those not detected by screening. Assuming that these tumors generally have a better prognosis than those not detected, the beneficial effect of screening could be artificially inflated. This appears to be a problem in the neuroblastoma screening studies to date. Cases with good prognostic markers are being detected by screening at 6 months rather than cases with poor prognostic markers. As Goodman (54) correctly noted, however, it is important to consider what is currently known about the biology of a disease when evaluating length-time bias. For example, in the case of neuroblastoma, what if the good biology tumors could evolve into bad biology tumors? Then, the observed pattern might not provide evidence for length bias. This has been addressed by Brodeur *et al.* (41), who demonstrated that, of 60 patients who were being treated for neuroblastoma, none had an MYCN amplification change during relapse or progression. This suggests that it is unlikely that neuroblastoma progresses from good to bad biology.

Finally, in detection bias, cases who might never have presented clinically are diagnosed in a screening program. To evaluate this bias, age-specific and overall incidence rates can be compared between screened and unscreened populations. Recent data from the Quebec study as well as from Japan indicate that the incidence of neuroblastoma in children less than 1 year of age dramatically increased after the introduction of screening programs. There has been no substantial reduction in the incidence of the disease in older children.

Conclusions

On the basis of the experience of neuroblastoma screening studies thus far, it is apparent that screening has inflated the incidence of neuroblastoma in children younger than 1 year and, generally, has had no effect on the incidence or survival of children diagnosed at ≥1 year old. The most logical explanation for this is that there are at least two and perhaps more distinct categories of disease, with probably little movement between them.

In summary, the following conclusions can be reached from the neuroblastoma screening studies. (*a*) Early screening

for neuroblastoma is feasible due to good compliance. Due to the biological nature of the tumors picked up by screening at 6 months of age, however, there is no reduction in mortality. (*b*) Screening at later ages may be appropriate, although there is likely to be a reduction in compliance. Moreover, it is possible that poor prognosis tumors develop so rapidly that the majority will be missed by screening at any given arbitrary age. (*c*) Before mass screening is implemented, a population-based study with an appropriate control group should be conducted to determine the effect of a screening program on mortality. This is essential to understanding the natural progression of the disease and using screening effectively.

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