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Original Paper

Screening for Neuroblastoma is Ineffective in Reducing the Incidence of Unfavourable Advanced Stage Disease in Older Children

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Neuroblastoma exhibits many characteristics which would suggest that preclinical detection may improve outcome. The Quebec Neuroblastoma Screening Project was initiated to determine whether mass screening could reduce mortality in a large cohort of infants. All 476 603 children born in the province of Quebec during a 5-year period of time (1 May 1989 to 30 April 1994) were eligible for determinations of urinary catecholamine metabolites at 3 weeks and 6 months of age. Children with positive screening were referred to one of four paediatric cancer centres in Quebec for uniform evaluation and treatment. Standardised incidence ratios (SIRs) were calculated for neuroblastoma in Quebec and two comparable population-based controls during the same period of time using similar ascertainment procedures. Compliance with screening in Quebec was 91% at 3 weeks ($n = 425\,816$) and 74% at 6 months ($n = 349\,706$). Up to 31 July 1995 with a follow-up of the birth cohort of 15–75 months, 118 cases of neuroblastoma were diagnosed, 43 detected preclinically by screening, 20 detected clinically prior to screening at 3 weeks of age and 55 detected clinically after 3 weeks of age having normal screens ($n = 52$) or never screened ($n = 3$). Based on data from concurrent control populations, 54.5 cases of neuroblastoma would have been expected in Quebec during the study period for an SIR of 2.17 (95% CI 1.79–2.57, $P < 0.0001$). For the two control groups, the overall SIR was 1.00 (NS). SIRs for Quebec by age at diagnosis in yearly intervals show a marked increased incidence under 1 year of age (SIR = 2.85, 95% CI 2.26–3.50), with no reduction in incidence in subsequent years. We conclude that screening for neuroblastoma markedly increases the incidence in infants without decreasing the incidence of unfavourable advanced stage disease in older children. It is unlikely that screening for neuroblastoma in infants will reduce the mortality of this disease. © **1997 Published by Elsevier Science Ltd.**

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INTRODUCTION

NEUROBLASTOMA IS the most common solid tumour of children younger than 5 years of age, cumulatively affecting

approximately 1 in 7000 individuals [2, 3]. Despite major advances in the treatment of other childhood cancers, the improvement in overall outcome for neuroblastoma in the last 15–20 years has been modest [3–6]. The potential that neuroblastoma could be detected by screening has been investigated in pioneering work by several Japanese investigators, who documented that neuroblastoma can be diagnosed preclinically with almost uniformly favourable survival [7–11]. There were early reports concerning the apparent success of the neuroblastoma screening studies in Japan. However, several methodological limitations, including the lack of controlled, population-based studies with adequate ascertainment, precluded a conclusion that the preclinical detection of this disease will reduce mortality [12–15].

The Quebec Neuroblastoma Screening Project was undertaken to determine whether screening a large birth cohort of infants for neuroblastoma could reduce the population-based incidence of advanced disease or mortality. Utilising the infrastructure of the Quebec Network for Genetic Medicine in which over 90% of all infants born in the province are screened by urine at an early age for various inborn errors of metabolism [16], screening for neuroblastoma was instituted for a 5-year birth cohort after preliminary studies were performed [17–20]. Province-wide incidence and mortality results are being compared with several population-based control groups in North America in which infants were not screened [21–23]. In this paper we report the effect of screening on the overall incidence of neuroblastoma in Quebec, and on the incidence of advanced disease.

MATERIALS AND METHODS

Description of the Quebec Neuroblastoma Screening Project

Specific aspects of the study design have been published in detail previously [18–23]. Briefly, with informed consent, infants born in the province of Quebec during a 5-year period (1 May 1989 to 30 April 1994) had urine samples tested at both 3 weeks and 6 months of age for elevated catecholamine metabolites—vanillylmandelic acid (VMA) and homovanillic acid (HVA). The screen at 3 weeks of age was performed on the urine sample used for screening for metabolic diseases, with a compliance rate of 91%. The second screen at 6 months was a newly initiated test. Filter papers were provided to the parents at birth and multiple reminders were given through various sources [21–24]. Compliance with the second screen was 74%.

Once a urine-saturated filter paper was received by the screening centre, thin layer chromatography (TLC) analysis for catecholamine metabolites was performed using a highly sensitive method [18–22]. Parents of infants with insufficient or contaminated samples were contacted for repeat testing. Approximately 5.3% of samples for the 3-week screen and 8.6% for the 6-month screen had a borderline or positive TLC result. These were sent to Minneapolis where the highly sensitive and specific gas chromatography–mass spectrometry (GC–MS) assay was performed for VMA and HVA [17, 21, 25]. Parents of children with abnormal GC–MS results were contacted by personnel in Sherbrooke and a second filter paper requested for GC–MS analysis. Patients with positive second analyses were referred to one of four PQ paediatric cancer centres for the evaluation of neuroblastoma using a prescribed algorithm, with specific follow-up required for patients in whom no neuroblastoma was found [21, 22].

Ascertainment of neuroblastoma in Quebec

The experimental cohort represented all children born in Quebec during the 5-year period of time noted above. Ascertainment of children with neuroblastoma, either diagnosed preclinically through screening, missed by screening or never screened, was accomplished by several methods [3]. Traditionally, well over 90% of all patients with neuroblastomas diagnosed in the province have been referred to one of the four provincial paediatric cancer centres. Close contact was established between study investigators and Canadian medical centres outside Quebec where patients may have received care.

All patients diagnosed with neuroblastoma in Quebec underwent uniform staging by the Evans' method [26], Pediatric Oncology Group (POG) staging [27] and the International Neuroblastoma Staging System [28, 29]. Four study investigators routinely reviewed the staging of each Quebec neuroblastoma patient, with difficult cases staged by consensus. Samples for specific biological parameters known to have prognostic importance in neuroblastoma, including *MYCN* gene copy number, DNA ploidy, *trk* gene expression, serum ferritin, catecholamine metabolites and histology, were collected in over 98% of all children diagnosed. Patients with neuroblastoma were subsequently treated based on age, stage and biological features on specific POG protocols open during the study period (POG studies 8105, 8741–8743, 8844, 9140, 9243, 9244, 9248 and 9340–9342).

Control groups

Two population-based non-screened control groups were followed during the same 5-year period: (1) *the state of Minnesota*, using Children's Cancer Group (CCG) data and the population-based, pathology-based Minnesota Cancer Surveillance System [30]; and (2) *the province of Ontario*, because of its geographic proximity with Quebec and, using the Pediatric Oncology Group of Ontario data [31]. Furthermore, the incidence and mortality of neuroblastoma in Quebec had been compared to data from the Greater Delaware Valley (GDV), the largest population-based paediatric tumour registry in North America [32], for a 10-year period of time (1977–1986) with similar outcomes found [3]. All patients diagnosed with neuroblastoma in the concurrent control groups were staged by the three systems noted above and appropriate data gathered.

Statistical and other considerations

Utilising 1-year age-specific incidence rates from the U.S. Surveillance, Epidemiology and End Results (SEER) programme [33, 34], standardised incidence ratios (SIRs) and corresponding 95% confidence intervals were calculated for Quebec, Minnesota and Ontario. SEER incidence rates for Caucasians only were used, since greater than 95% of all three populations are Caucasian. Stage-specific incidence rates obtained from the GDV paediatric tumour registry were utilised for calculation of SIRs within age- and stage-specific subgroups because the SEER programme does not collect data on neuroblastoma staging.

Quebec, Minnesota and Ontario patients were recorded as having neuroblastoma if they lived in the respective area when they were diagnosed with the disease and were born between 1 May 1989 and 30 April 1994 (5 years), irrespective of place of birth. In- and out-migration were expected to be low in Quebec based on previous census data from the

province, with only two cases of neuroblastoma expected to emigrate out and three additional cases of neuroblastomas expected by immigration during the study period (G. Dougherty, Montreal Children's Hospital, Montreal, Canada). In reality, 3 patients were diagnosed having emigrated from Quebec after birth (2 detected by screening, 1 never screened), and no patients were diagnosed while residents of Quebec having been born elsewhere. These 3 patients are not included in the Quebec incidence analysis.

Staging was compared between Quebec, Minnesota and Ontario based on concurrent ascertainment of cases, with data tabulated utilising the INSS. As an additional control, incidence of Evans' early (I, II, IVS) and advanced (III, IV) disease in patients under and over the age of 1 year was determined for the three concurrent study groups and compared with updated, previously acquired and reported GDV data, obtained at a time when only Evans' staging was utilised [3]. Although the staging data for the GDV retrospective population were not reviewed, our previous study documented comparable results to the retrospective population of Quebec where staging was performed by the same investigators participating in the current study.

RESULTS

Cases of neuroblastoma diagnosed in Quebec

Up to 31 July 1995, with a follow-up of 15–75 months, there have been 118 cases of neuroblastoma diagnosed among the birth cohort in Quebec. Forty-three patients were detected preclinically with neuroblastoma by screening, 17 at the 3-week screen and 26 at the 6-month screen. The overall positive predictive value of the screening approach was 52% (43 of 82). The INSS neuroblastoma staging of Quebec cases diagnosed through screening is summarised in Table 1. All patients detected by screening are alive and disease-free.

During this same study period, 55 patients were detected clinically after 3 weeks of age, having been undetected by screening ($n = 52$) or never screened ($n = 3$). Only 3 patients in the entire cohort had positive tests by GC–MS retrospective analysis of the six-month samples. Two children were diagnosed at 7 and 8 months with localised tumour and are well. Only 1 child was missed by the screening procedures who presented clinically over 1 year of age, a 53-month old diagnosed with stage 4 neuroblastoma who, in retrospect, had an elevated HVA level by GC–MS at the 6-month screen but had a normal TLC analysis (probably a dilute urine sample). All 49 other children missed by screening could clearly not have been detected at the two screening periods as they were non-secretors at the time of screening.

Table 1. *INSS staging of neuroblastoma cases in Quebec for the* 5-*year screened cohort* (n = 118)

INSS stage	Cases diagnosed prior to screening at 3 weeks of age	Cases diagnosed through screening	Cases missed by screening or never screened	Total
1	10	10	14	34
2A	1	0	2	3
2B	0	11	7	18
4S	6	8	3	17
3	1	6	5	12
4	2	8	24	34
Total	20	43	55	118

An additional 20 neuroblastoma patients were detected clinically before 3 weeks of age. All were catecholamine secretors and 7 actually had submitted urines to the screening programme when they were diagnosed. Nineteen were detected by routine neonatal physical examinations, chest x-rays or ultrasounds performed for incidental reasons (including two performed prenatally). One was detected as an incidental finding at surgery performed for other reasons.

Incidence of neuroblastoma in Quebec and in the control groups

Using incidence data from the SEER programme of the United States National Cancer Institute, as described above, the number of expected neuroblastoma cases was calculated in Quebec, Ontario and Minnesota for the 5-year birth cohort. Based on SEER data, 54.5 cases of neuroblastoma were expected to be diagnosed in Quebec children born during the cohort period, whereas 118 cases have been observed thus far. The SIR was 2.17 (95% confidence intervals (CI), 1.7–2.57, $P < 0.0001$ Table 2). Table 2 also lists the size of the 5-year cohorts for the two control groups and the number of observed and expected neuroblastoma cases. Remarkably, the SIRs for the combined control groups was 1.00, with both groups individually showing no statistically significant changes in SIRs.

SIRs were further calculated for Quebec and the two control cohorts by age at diagnosis in yearly intervals (Table 2). For Quebec, the observed to expected ratio of cases diagnosed with neuroblastoma under 1 year of age was significantly higher than that expected (SIR of 2.85; 95% CI of 2.26–3.50). For each of the subsequent yearly periods, there was no significant decrease in the SIR (Table 2). Furthermore, no statistically significant increased or decreased incidences were seen in either Ontario or Minnesota for any of the six age groups studied.

Neuroblastoma staging in the Quebec and control cohorts

In order to examine whether screening had any impact on the relative distribution of various neuroblastoma stages, INSS results were contrasted among Quebec, Minnesota and Ontario. The number of patients in the control groups were 'normalised' to the Quebec population by comparing the number of births for the 5-year cohorts. The number of patients diagnosed with early stage disease in Quebec, especially stage 1, was far in excess of that seen in the control groups. However, no concomitant decrease was noted in the more advanced stages (3 and 4), as would have been expected if preclinical detection of neuroblastoma had reduced the incidence of late-stage disease. In fact, the number of cases with stage 4 disease diagnosed in Quebec [34] was substantially greater than that seen in the normalised control groups (25.9 and 15.9 in Minnesota and Ontario, respectively), with only a portion of the excess in Quebec seen in children under 1 year of age by screening (Table 2).

Finally, the SIRs of early and advanced Evans' stage neuroblastoma in infants and older children were calculated for the screened and control cohorts utilising GDV data (Table 3). The SIR for the entire Quebec cohort was markedly elevated compared to GDV, 2.39 (1.98–2.84), with Minnesota and Ontario showing no significant increases or decreases (Table 3). Forty-five advanced stage (Evans' III and IV) cases were diagnosed during the study period in Quebec, versus 26.0 expected (SIR = 1.73; CI of 1.26–2.28). For the critical group with advanced stage disease diagnosed over 1 year of

Table 2. Standardised incidence ratios for neuroblastoma at 1-year age intervals comparing the Quebec cohort with the control populations (Minnesota and Ontario) utilising U.S. SEER data

Age at diagnosis	Quebec	Minnesota	Ontario
0–11 months			
Person years	476 172	331 480	747 996
Observed	81	17	40
Expected	28.48	19.82	44.73
SIR	2.85	0.86	0.89
95% CI	2.26–3.50	0.50–1.31	0.64–1.19
12–23 months			
Person years	448 248	311 864	703 170
Observed	20	13	24
Expected	12.69	8.83	19.90
SIR	1.58	1.47	1.21
95% CI	0.96–2.34	0.78–2.38	0.77–1.74
24–35 months			
Person years	358 234	143 797	558 594
Observed	8	8	7
Expected	8.31	5.77	12.96
SIR	0.96	1.39	0.54
95% CI	0.41–1.74	0.59–2.51	0.21–1.01
36–47 months			
Person years	262 540	183 087	408 088
Observed	5	4	5
Expected	2.99	2.09	4.65
SIR	1.67	1.92	1.08
95% CI	0.53–3.46	0.50–4.25	0.34–2.22
48–59 months			
Person years	164 885	116 261	256 676
Observed	2	1	4
Expected	1.73	1.22	2.70
SIR	1.16	0.82	1.48
95% CI	0.11–3.31	0.00–3.21	0.39–3.30
60–71 months			
Person years	45 291	32 606	71 219
Observed	2	0	0
Expected	0.30	0.22	0.47
SIR	6.69	—	—
95% CI	0.63—19.18	—	—
Totals			
Births	476 603	331 425	748 532
Person years	1 755 460	1 119 095	2 745 743
Observed	118	43	80
Expected	54.50	37.94	85.41
SIR	2.17	1.13	0.93
95% CI	1.79–2.57	0.82–1.50	0.74–1.14

age, no decrease was observed (22 cases detected in Quebec versus 14.4 expected; SIR = 1.52; CI of 0.95–2.23). In contrast, neuroblastoma incidence in the two control groups was similar to that expected. There was no evidence of an increase or decrease in any stage disease in children under or over 1 year of age except for an increase in early stage disease in children over the age of 1 year in Minnesota (10 versus 3.75 expected, SIR = 2.67 [1.27–4.58]) (Table 3).

DISCUSSION

Neuroblastoma exhibits several features which at first glance would suggest that preclinical detection may reduce mortality. First, neuroblastoma has been hypothesised to be an embryonal neoplasm which presents with decreasing frequency from birth to 5 years of age. When infants are diagnosed with the disease, the tumour is often localised, exhibits favourable biological characteristics and has an excellent prognosis, even when diagnosed with metastatic disease (stages 4 or 4S) [35, 36]. However, children diagnosed over the age of 1 year have a distinctly poor outcome, with the majority presenting with widely disseminated disease and resistance to aggressive therapy, including myeloablative treatment followed by bone marrow transplantation rescue [4, 44–46]. Second, catecholamine metabolites are well known to be excreted in the urine of most children with neuroblastoma, and they are easily detectable by several quantitative and qualitative methods [17, 18, 24, 25, 40]. Between 70% and 90% of all neuroblastomas secrete VMA—a metabolite of noradrenaline, or HVA—a metabolite of dopamine [41, 42]. Third, as noted above, the overall outcome for neuroblastoma patients has lagged behind that of other common paediatric neoplasms [3–6].

As the Quebec project progressed, it became increasingly clear that the introduction of screening profoundly changed the natural history of the disease in the population, resulting in more than a 2-fold increase in incidence. The increased incidence was most dramatic for infants less than one year of age during the screening period, in which the SIR was 2.85

Table 3. Standardised incidence ratios of early and advanced Evans' stage neuroblastoma comparing the Quebec cohort with the control populations (Ontario and Minnesota), utilising greater Delaware Valley data

Age	Quebec			Minnesota			Ontario		
	< 1 Year	≥ 1 Year	Total	< 1 Year	≥ 1 Year	Total	< 1 Year	≥ 1 Year	Total
Early stage*									
Observed	58	15	73	11	10	21	25	10	35
Expected	19.12	5.37	24.49	13.36	3.75	17.11	30.47	8.42	38.89
O/E	3.03	2.80	2.98	0.82	2.67	1.23	0.82	1.19	0.90
95% CI	2.30–3.86	1.56–4.39	2.34–3.70	0.41–1.38	1.27–4.58	0.76–1.81	0.53–1.17	0.57–2.04	0.63–1.22
Advanced stage†									
Observed	23	22	45	6	16	22	15	30	45
Expected	11.53	14.44	25.97	8.05	10.08	18.13	18.37	22.67	41.04
O/E	2.00	1.52	1.73	0.76	1.59	1.21	0.82	1.32	1.10
95% CI	1.26–2.89	0.95–2.23	1.26–2.28	0.27–1.46	0.91–2.46	0.76–1.77	0.46–1.28	0.89–1.84	0.80–1.44
All stages									
Observed	81	37	118	17	26	43	40	40	80
Expected	30.65	18.78	49.43	21.41	13.83	35.24	48.84	31.09	79.93
O/E	2.64	1.97	2.39	0.79	1.88	1.22	0.82	1.29	1.00
95% CI	2.10–3.25	1.39–2.66	1.98–2.84	0.46–1.22	1.23–2.67	0.88–1.61	0.59–1.09	0.92–1.72	0.79–1.23

*Early stage: Evans' stage I, II, IVS. †Advanced stage: Evans' stage III, IV.).

(95% CI 2.26–3.50). This would have been considered a desired effect if the incidence of neuroblastoma had decreased in older children, but such was not the case. SIRs for the period from 1 to 5 years show an increase in the incidence of neuroblastoma with no decreases noted in: (i) advanced stage disease at any age; (ii) overall disease in children over 1 year; or (iii) advanced stage disease in patients older than 1 year, in whom prognosis can be expected to be poor [2–5, 36–39] (Tables 2 and 3).

Changes noted in the incidence of neuroblastoma in Quebec were not seen in the two concurrent population-based control cohorts in Minnesota and Ontario which had similar neuroblastoma ascertainment procedures and staging review as Quebec. Furthermore, the annual neuroblastoma incidence in Quebec during the screening period, approximately $66/10^6$ for the 0–4 age group as calculated from our data, is 2.4-fold higher than in Quebec prior to screening ($28/10^6$) [3].

Based on early reported data, we and others have hypothesised that neuroblastoma represents at least two distinct clinical-biological entities [43, 49]. The first is favourable neuroblastoma, which is probably congenital, is associated with young age and early stage at diagnosis, triploid karyotypes, no 1p abnormalities or *MYCN* gene amplification, more mature catecholamine synthesis and excretion and excellent clinical outcome despite no or minimal therapy. Increasing evidence from our study and from those in Japan [7–11] suggests that favourable neuroblastoma can be easily detected through screening for catecholamine metabolites, but their overall outcome is excellent even after clinical detection. The second clinical-biological entity, unfavourable neuroblastoma, presents at an older age, generally with advanced stage, pseudodiploid karyotypes, 1p deletions, *MYCN* oncogene amplification, less mature catecholamine synthesis and excretion and poor outcome. The data reported herein and suggested elsewhere [44, 45] document that screening for neuroblastoma at or before 6 months of age does not reduce the incidence of unfavourable neuroblastoma, based on age and stage at diagnosis. Overall results of biological features which will be reported elsewhere, confirm that characteristics of neuroblastomas detected by screening, or clinically detected prior to 1 year of age, are generally favourable, while older children detected clinically generally have neuroblastomas with unfavourable biology [46–48]. It also appears there is an intermediate group that has some clinical and biological features of unfavourable neuroblastomas, but lacks *MYCN* amplification. These patients generally have a more indolent course, but their overall prognosis is still poor.

It is well known that neuroblastomas may spontaneously regress [13, 50]. Quantitation of regressing neuroblastomas has been impossible prior to the Quebec Neuroblastoma Screening Project. From population-based studies of neuroblastoma incidence over the last 30–50 years, there has been a suggestion of a slow increase in the incidence of the disease [51, 52]. We think that a substantial proportion of this increased incidence is due to better clinical detection such as through the use of more sensitive diagnostic imaging rather than a true increase in the disease.

The results of this study also demonstrate a striking 'halo' effect from the neuroblastoma screening programme in which the overall incidence of neuroblastoma was increased in Quebec, even if one excludes patients detected by screening. Multiple public relations and public health methods aimed at improving compliance with screening were implemented in Quebec during the screening period. Increased awareness of neuroblastoma in the entire province by health care providers apparently led to more cases diagnosed than would have been otherwise. This increase was quite apparent in the neonatal period, where 20 cases of neuroblastoma were detected prior to screening at 3 weeks of age. However, there was a significant increase in neuroblastomas diagnosed over 1 year of age as well (Table 3). Other investigators have hypothesised a similar reason for discrepant neuroblastoma incidences in various developed countries [53]. Many children currently clinically detected in developed countries with neuroblastoma may have escaped detection in years prior to the increased use of perinatal imaging technologies. These effects raise the issue of whether children who are diagnosed with favourable neuroblastoma, especially at an early age, need any treatment for their tumours [48, 54]. Neuroblastomas with favourable

biologic features rarely, if ever, evolve into unfavourable disease [55].

Utilising state-of-the-art methodology and a compliance rate comparable to that in Japan, we were able to demonstrate conclusively in the Quebec Project that the majority of children who currently do poorly after being clinically diagnosed with neuroblastoma over 1 year of age cannot be detected preclinically by screening at or before 6 months. Although it has been previously assumed that all neuroblastomas are present *in utero*, this appears not be the case. Even if some neuroblastomas are embryonal, many of these are relatively quiescent *vis-á-vis* size and/or catecholamine secretion and are hard to detect until after a year of age. Although the mortality assessment in the Quebec Project awaits longer follow-up, we contend that the incidence data reported herein support the conclusion that screening for neuroblastoma in infants at or before the age of 6 months will not reduce mortality from this disease. Widespread screening of neuroblastoma during this time period therefore should not be adopted.

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