Pergamon

European Journal of Cancer Vol. 31A, No. 4, pp. 565–568, 1995

0959-8049/95 $9.50+0.00

0959-8049(95)00034-8

Neuroblastoma Mass Screening: The Arguments For and Against

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Neuroblastoma is the second commonest malignancy in childhood. The prognosis of the disease is largely dependent on the extension of the tumour at diagnosis. For disseminated disease the survival rate is very low. The question as to whether mass screening in infants can improve the prognosis of the disease was first addressed in Japan more than 20 years ago. Since then, more than 7 million children have been screened in Japan and over 650 cases of neuroblastoma have been detected. However, the available data are compromised by an inadequate cancer registry and conclude that screening at 6 months of age seems to double the incidence of neuroblastoma. This result has been verified by a Canadian study conducted from 1989 to 1994 in the province of Quebec. The incidence of neuroblastoma appeared to have tripled, and there was no decrease in the rate of advanced disease. Mass screening pilot studies have also been conducted in the U.K., France, Austria, Australia, U.S.A., Italy, Norway and Germany. Analysis of the results shows that neuroblastoma screening before the age of 6 months is feasible, but no significant reduction in mortality could be shown until now. Moreover, most of the cases diagnosed by screening have favourable biological markers. Only a few with unfavourable parameters, such as amplification of proto-oncogene *MYCN*, diploidy and/or del 1p36 could be detected. A screening programme that includes 1.25–2 million screened and unscreened children at 1 year of age monitored by an almost complete national cancer registry should show whether mass screening for early detection of neuroblastoma is worthwhile.

Key words: neuroblastoma, mass screening, biology
Eur J Cancer, Vol. 31A, No. 4, pp. 565–568, 1995

INTRODUCTION

THE QUESTION as to whether neuroblastoma screening in infants is the correct approach in attempting to improve the prognosis of neuroblastoma cannot at present be answered with any degree of certainty. Central to the discussion is the clinical experience with localised neuroblastoma. In general, the prognosis for neuroblastoma in infants is much more favourable than in children diagnosed after the first year of age when the disease often presents at a more advanced stage. Alternatively, the results of the biological and molecular biological research in neuroblastoma in recent years suggest that there are two different entities, one with a favourable prognosis and one with an unfavourable prognosis, possibly from the onset of the disease. The available data from the neuroblastoma screening programmes in Japan and Canada seem to support the concept of overdiagnosis of neuroblastoma with a favourable prognosis. An attempt will be made to summarise the current state of knowledge of the value of neuroblastoma screening, and to try to answer the question as to what the next step should be.

CLINICAL ASPECTS

Neuroblastoma is the second commonest malignancy in childhood. It arises in the adrenal medulla and peripheral sympathetic nervous tissue. The tumour is often situated in an anatomical region which does not allow for early diagnosis. The annual incidence in Germany is 1 in 100 000 children under the age of fifteen, which is comparable to the incidence of inborn errors of metabolism. As approximately 90% of cases of neuroblastoma present within the first 2 years of life, the incidence in the first year is 6 in 100 000, and after the first to the fifth year is 1.7 in 100 000 [1]. The prognosis for children with neuroblastoma is largely influenced by the extension of the disease at diagnosis. The chance of cure for localised disease (Evans stage I and stage II) is 92%, while the long term survival for wide localised disease (stage III) is 60%. For disseminated disease at diagnosis, the survival rate falls to 14%. Only approximately 20% of neuroblastoma can be detected in localised stages (without screening). Approximately 80% of all neuroblastoma cases present with regional spread to lymph nodes or have metastases in bone or bone marrow at diagnosis [2]. From these data and the assumption that neuroblastoma, like other malignant solid tumours, progresses from a low to a higher stage, it follows that there is interest in trying to diagnose the disease at an early stage. In addition, disseminated neuroblastoma diagnosed within the first year of life has a more favourable prognosis as compared to the older child [3]. Most of the tumours are able to produce catecholamines, the metabolites of which are excreted in urine. The catecholamine metabolites vanillylmandelic acid (VMA) and homovanillic acid (HVA) can easily be detected by sensitive methods which raises the possibility of early detection. In an analysis by Berthold and associates [4], 101 children with

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asymptomatic neuroblastoma were detected by chance from an unscreened population. Of the 101 patients, 49.2% had stage I, 30% stage II, 17.6% stage III and 5.6% stage IV disease. The distribution of stage is the reverse of the pattern seen for symptomatic neuroblastoma. A further observation that supports the hypothesis that neuroblastoma develops from localised to advanced disease is that the median age at diagnosis also increases with stage. The study by Berthold and associates showed a median age of 7 months for stage I, 7.5 months for stage II, 20 months for stage III and 34 months for stage IV. The clinical data suggest but do not prove that localised neuroblastoma (stage I and II), when not diagnosed asymptomatically, will develop into a more advanced stage III and IV tumour with increasing age.

RESULTS OF MASS SCREENING TRIALS

Screening for neuroblastoma in Japan

The group from Sawada in Kyoto/Japan first performed neuroblastoma mass screening as a feasibility study in 6-month-old children [5]. The method used was a qualitative spot test to measure the VMA level from a spontaneous urine sample. In 1981, a national screening programme was introduced as a pilot study carried out in nine regions in Japan. Simultaneously, Taketa and his co-workers introduced an early detection programme using a high pressure liquid chromatography method (HPLC). A national screening programme, using the HPLC method, was started in Japan in 1985 to examine the urine of all 6-month-old infants. By the end of 1989, 337 cases of neuroblastoma in an early stage had been detected [6]. However, the results of the national Japanese study, carried out in approximately 7 million children, between 1985 and 1992, with over 650 cases detected, are not conclusive as to whether the number of cases of stage IV disease can be reduced and the survival rate for neuroblastoma improved [7]. The reasons for this are 2-fold. There is a totally inadequate cancer registry in the country, and there is no national consensus or agreed policy about the treatment of neuroblastoma in Japan. The deficient registration system leads to an inaccurate analysis of the survival and mortality rates. However, the available data do conclude that, in the regions where screening is performed, the incidence of neuroblastoma has doubled from 4.6 per million in 1980 to 8.39 per million children in 1988. As a comparison, the incidence of neuroblastoma in other countries is not much lower at 6.6 per million [8]. The observed increase in the incidence of neuroblastoma after the start of screening can also be explained by an improvement in the registration of cases.

Results of screening in Quebec

The Canadian study carried out a screening programme between 1989 and 1994 of all babies at 3 weeks and 6 months of age in the Quebec region. The method used was thin layer chromatography, and gas chromatography/mass spectrometry was performed on all positive results. The control groups for the study were the district of Ontario, Florida, Minnesota and the Greater Delaware Valley. Until August 1993, a total of 368 000 babies aged 3 weeks and 269 000 aged 6 months were tested. Out of the total population of the Quebec region, 79 cases were asymptomatic and detected by the screening programme. The remainder were detected clinically, 17 before the third week of age, 10 between 3 weeks and 6 months and 20 after 6 months. From the 20 symptomatic cases of neuroblastoma diagnosed after 6 months of age, 18 had a negative screening test at 3 weeks and 14 proved negative at 6 months. 4 patients had not been screened at the age of 6 months. 14 of the 20 patients presented with more advanced stage III and stage IV disease [9]. The design of the Canadian study, with two examinations, has only resulted in even more difficulty in statistical interpretation of the results, with regard to the relevance of neuroblastoma screening. The current impression is that screening leads to overdiagnosis of neuroblastoma, and does not influence the rate of advanced disease in the screened and control populations.

Results of screening in the North of England

A pilot study was initially introduced as a feasibility study in the North of England between 1985 and 1990. During this period, 22 000 babies were screened at the age of 6 months. 2 cases of neuroblastoma were detected in a screened population of 21 000. In addition, 3 children developed stage IV neuroblastoma, all of whom had a negative catecholamine screening test at 6 months. The incidence of neuroblastoma with 5 detected cases was 1 in 4100 compared with the previous incidence of 1 in 10 000 up to the age of 15 years [10]. Owing to the small numbers used no epidemiological conclusion can be drawn from this study.

Neuroblastoma screening in Lyon, France

In the Rhone district of Lyon, a feasibility study was started in 1990 and up to end of 1994, 84 000 babies aged 4 months had been tested. The total number of births for this region was 103 000. 10 cases of neuroblastoma were found, 4 stage I, 2 stage II, 1 stage III and 3 stage IV. 4 additional cases were detected earlier than the fourth month of life. There were 2 cases of neuroblastoma in whom the screening test had been negative at 4 months. One presented at the age of 6 months with stage III disease and one at 24 months with metastatic disease. The incidence in the population of 100 000 children is 1 in 5800 compared with the historical incidence of 1 in 8400. In this study, neuroblastoma also appears to be overdiagnosed as a result of routine screening. However, the numbers are somewhat uncertain as the incidence in the control group in the Auvergne in the same time period was 1 in 4500 [11].

Neuroblastoma screening in Germany

A feasibility study was carried out in the areas of Stuttgart and Hamburg from 1991 until 1994. Over 36 months, urine samples from almost 100 000 children aged 6 months were examined. 11 cases of asymptomatic neuroblastoma with a mean age at diagnosis of 9.7 months were detected. 6 had stage I, 2 stage II, 1 stage III, 1 stage IV and 1 stage IVS according to the Evans's staging system [12]. Another pilot study began at the end of 1992, testing children at the age of 12 months in Northrhine-Westphalia and Lower Saxony.

Other pilot projects

A number of other pilot studies are currently being carried out in Graz (Austria), Houston, Texas (U.S.A.), Birmingham (U.K.), Oslo (Norway), Rome (Italy) and New South Wales (Australia).

BIOLOGICAL AND MOLECULAR BIOLOGICAL ASPECTS

The biological markers of neuroblastoma detected within the last few years are linked to prognosis. The advanced cases and prognostically unfavourable neuroblastoma are correlated with specific cytogenetic markers. Diploid neuroblastoma with and without chromosomal changes, the finding of chromosome 1p

deletion, *MYCN* amplification, and the failure to express nerve growth factor receptor gene *TRKA* are parameters which are linked to more advanced disease [13]. In contrast, hyperdiploid neuroblastomas with few cytogenetic changes and a high expression of nerve growth factor receptor are associated with a favourable prognosis, and are often correlated with lower stages [14]. There are two essential questions which arise when one looks at the biological markers with respect to neuroblastoma screening. What are the cytogenetic characteristics of the neuroblastomas which are detected as a result of mass screening? Can neuroblastoma with favourable molecular biological features develop into a tumour with unfavourable markers in the course of time. The majority of cases detected at 6 months by mass screening have been hyperdiploid tumours without structural abnormalities, such as 1p deletion, double minutes, or homogeneous staining regions. Usually neuroblastomas detected by mass screening do not show evidence of *MYCN* amplification [15]. Exceptions have been found in the German (Hamburg–Stuttgart) [16] and Austrian programmes [17]. Within the German pilot trial, 2 of 11 cases of neuroblastoma detected by screening had unfavourable biological markers. One child with stage I disease had a diploid tumour with 1p deletion and *MYCN* amplification, while another case demonstrated diploidy with 1p deletion without *MYCN* amplification. In summary, it can only be assumed that neuroblastoma detected by mass screening can be considered as having a favourable prognosis according to the defined molecular biological criteria. The question remains, however, as to what extent the neuroblastoma with biological favourable markers can progress to disease with unfavourable markers. It is also conceivable that a small proportion of cases of neuroblastoma detected by screening has, from the onset, a poor prognosis, but owing to early detection the clinical outcome is probably good.

CONCLUSIONS

On the basis of the problems outlined above, questions with regard to neuroblastoma arise. From the results of the earlier screening programmes, it can be concluded that it is possible to detect asymptomatic neuroblastoma cases, and there is a suggestion that early screening at the age of 6 months or earlier can result in overdiagnosis of neuroblastoma, which means that more cases of the disease are diagnosed than would be manifested clinically. It is not yet possible to determine whether the incidence of stage IV neuroblastoma or the mortality from the disease can be reduced as a result of any neuroblastoma mass screening programme.

In order to reduce the problem of overdiagnosis of neuroblastoma, screening should be carried out later than 6 months, for example at 12 months. As a result, the trade-off would be that cases occurring earlier than 12 months would not be detected. However, this does not appear to be a problem as children presenting under 1 year have a favourable prognosis, which is independent of stage. This phenomenon of overdiagnosis is also an ethical problem because treatment in overdiagnosed cases could result in an increased morbidity and mortality in these children. When screening is postponed to the twelfth month of life, one can speculate that the false negative cases from Japan, Canada and England should be detected, if the tumour has the potential to secrete catecholamines. A complete cancer registry is also a very important requirement for a screening programme in order to follow historical controls as well as study and control populations. Also necessary is the uniformity of treatment of neuroblastoma.

The SENSE-Group (Study for the Evaluation of Neuroblastoma Screening in Europe) in cooperation with the International Agency for Research on Cancer (Lyon, France) are working on an enormous scale to develop a project which will eventually lead to some conclusions [18]. It will be necessary to test 3 million children in order to produce significant results. According to statistical power calculations for a trend analysis done by the Institute for Medical Statistics and Documentation in Mainz, Germany (J. Michaelis, personal communiction), a study which aims to reduce the cumulative mortality by 30% should include at least a population of 1.25 million tested 1-year-old children and a control group of 1.25 million who have not been tested, examined over 4–5 years and then followed up for a further 5 years. These requirements assume that there will be a compliance rate of over 80%. Attached to the screening, there should be a research programme to look at the biological and molecular biological characteristics of neuroblastoma detected by mass screening. This includes chromosomal analysis, for example, 1p deletion, the expression of *MYCN*, *TRKA* and CD44 adhesion molecule, DNA ploidy, etc. An important part of the characterisation of the disease is Shimada's histological classification and the biochemical parameters, neuron specific enolase (NSE), ferritin and lactate dehydrogenase (LDH).

The concept of a screening programme that includes between 1.25 and 2 million children aged 1 year and a similarly sized control group, together with a complete national children's cancer registry could most likely solve the dilemma as to whether neuroblastoma screening can lead to a reduction in the mortality of neuroblastoma, and whether it will be worthwhile to recommend this procedure for implementation in a preventative programme for children.

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European Journal of Cancer Vol. 31A, No. 4, pp. 568–571, 1995

0959–8049/95 $9.50+0.00

0959-8049(95)00032-1

Clinical Strategies for the Treatment of Neuroblastoma

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Neuroblastoma is the most common solid extracranial tumour in childhood. In spite of intensive efforts of clinicians and scientists the prognosis for advanced disease is still poor. This paper presents a short review of the state-of-the-art in conventional treatment including surgery, chemotherapy, and radiation. This is followed by a review of the treatment attempts with high dose chemotherapy followed by autologous bone marrow or stem cell transplantation. One of the main problems with this approach is the contaminating tumour cells. Finally the various immunotherapeutic strategies are summarised which are used to remove minimal residual disease. Later, our new approach, combining various treatment modalities, is described.

Key words: neuroblastoma, clinical strategies, high dose chemotherapy, immune therapy
Eur J Cancer, Vol. 31A, No. 4, pp. 568–571, 1995

INTRODUCTION

NEUROBLASTOMA IS the most common solid extracranial tumour in childhood. It shows a remarkable biological heterogeneity, ranging from spontaneous differentiation or regression to progression in spite of intensive multimodal therapy. According to clinical and radiological criteria, 4 stages were initially defined by Evans and associates [1]. Recently, the Forbeck classification has been introduced based on additional surgical and histological criteria [2]. The clinical outcome for lower stages of neuroblastoma is normally good, whereas the prognosis for advanced stage III and stage IV tumours is poor. Additional risk factors, such as elevated lactate dehydrogenase (LDH), resectability of primary tumour, age > 9 months at diagnosis, decreased white blood cell count, or the histological presence of undifferentiated tumour tissue, have been described [3]. Recently, additional biological features have been identified which may predict the clinical outcome [4–7]. Based upon DNA ploidy, chromosome 1p deletion, *MYCN* amplification, loss of heterozygosity (LOH), and expression of the high affinity nerve growth factor receptor p140^{trk} (*TRKA*) risk groups can be identified. Tumours within the "good risk group" are likely to differentiate or regress spontaneously, or respond favourably to chemotherapy. In the "bad risk group", aggressive multimodal therapy regimens or innovative therapeutic modalities are justified. All therapeutic modalities used in neuroblastoma should, therefore, be adapted to the stages and biological risk factors. This should prevent overtreatment of low risk neuroblastomas on the one hand, and undertreatment of high risk tumours on the other.

The aim of this review is to discuss risk-adapted therapeutic

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