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Original Paper

Survival from Non-Stage 4 Neuroblastoma Without Cytotoxic Therapy: an Analysis of Clinical and Biological Markers

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The clinical characteristics of 43 patients (pts) and the biological features of their non-stage 4 neuroblastoma (11, 3, 15, 7 and 7 with stages 1, 2A, 2B, 3 and 4S, respectively) all managed initially without cytotoxic therapy at Memorial Sloan-Kettering Cancer Center are summarised. We staged patients by the International Neuroblastoma Staging System and measured their urine and serum tumour markers. Tumour *MYCN* copy number, chromosomal ploidy, chromosome 1p deletion, Shimada histopathology, trk-A and CD44 expression were analysed. Among patients with localised tumour ($n = 36$), 13 had residual disease after initial surgery, 19 had regional lymph node invasion and 6 had epidural involvement (2 of 6 being paraplegic). All 7 stage 4S patients had liver tumours, 3 had bone marrow involvement and 3 had lymph node involvement. The most common adverse biological markers were unfavourable histopathology (9/40 evaluable tumours) and diploidy (7/39 tumours tested). At a median follow-up of 50+ months, 42 patients are alive and well (5 with evidence of disease), and 1 patient in remission died of encephalopathy. Progressive/recurrent disease occurred in 12 patients, 1 stage 2A, 2 stage 2B, 4 stage 3 and 5 stage 4S. Chemotherapy was eventually used in 4 patients: a 3-year-old stage 2B patient who developed stage 4; a 2-year-old whose recurrent tumour had poor-risk biological markers; a 1-year-old whose recurrent stage 3 disease infiltrated a vertebral body and a stage 4S infant with respiratory impairment from progressive hepatomegaly. Three of the treated patients had diploid tumours. We conclude that non-stage 4 is of itself a strong predictor of a favourable outcome. Diploidy, unfavourable histopathology and unresectable tumours were associated with disease progression. However, evolution of local-regional tumour into distant metastatic stage 4 disease is not typical of neuroblastoma. © **1997 Elsevier Science Ltd.**

Key words: neuroblastoma, local-regional, 45, progression histopathology, diploidy, *MYCN*, CD44, trk-A
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INTRODUCTION

SLIGHTLY MORE than 50% of neuroblastomas present with involvement of distant bone and bone marrow (stage 4); their outlook is poor even with intensive therapy. The remainder present with localised, regional or stage 4S disease [1]. Many of these children are treated with chemoradiotherapy for fear of tumour progression, i.e. evolution of the regional form to the malignant (often incurable) stage of distant metastatic disease. The presence of adverse clinical/biological features is often used to justify intensification of treatment modality. At least two types of adverse prognostic factors have been used. Adverse tumour variables include *MYCN* amplification [2, 3], absence of trk-A expression [4, 5], deletion of chromosome 1p36 region [6–8], expression of the multidrug-resistant-protein (MRP) [9] and near diploidy/near tetraploidy [10, 11]. Adverse clinical–pathological risk factors include age of more than 1 year at diagnosis, local lymph

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node invasion, residual tumour after surgical resection, epidural tumour extension, size and origin (abdominal) of the primary tumour, elevated serum ferritin, LDH (lactate dehydrogenase) > 1500 U/l, elevated neuron-specific enolase (NSE), poor-risk histopathology by Shimada's or Joshi's criteria, capacity of tumour cells to grow continuously in culture and low VMA (vanillyl mandelic acid)/HVA (homovanillic acid) ratio in urine [1]. Tumour recurrence, even when local, is often used as an index of malignancy which requires intensive chemoradiotherapy. Despite the repeated observations of spontaneous remissions and excellent survival among local regional/stage 4S disease, the biology of neuroblastoma is often viewed as a continuum where cytotoxic therapy is tailored to individual risk factors.

Previously we reported the excellent survival of patients with local non-stage 4 neuroblastoma who received no cytotoxic therapy [12]. Here we have extended these studies and summarised the clinical/pathological as well as tumoral biological features of all the non-stage 4 patients treated over a 10-year period at Memorial Sloan-Kettering Cancer Center, New York, U.S.A.

MATERIALS AND METHODS

From September 1986 to April 1996, 43 consecutive patients with newly diagnosed non-stage 4 neuroblastoma were studied at the Memorial Sloan Kettering Cancer Center. Patients were evaluated by computed tomography (CT), ^{99m}technetium bone scan, ^{131}I-*meta*-iodobenzylguanidine scan, bilateral bone marrow aspirates and biopsies and measurement of urinary and serum tumour markers. At diagnosis, patients underwent surgical biopsy, debulking or resection without jeopardising vital organs. During surgery, particular attention was paid to the liver and regional lymph nodes. International Neuroblastoma Staging System (INSS) criteria were used to establish the diagnosis and to assign a clinical stage [13, 14]. Newly-diagnosed non-stage 4 patients received no cytotoxic therapy. If bony metastatic disease developed, or tumour enlargement caused life-threatening mechanical obstruction or compromise of major organs, patients were treated with appropriate chemotherapy regimens [15].

MYCN amplification (>10 copies) was determined by Southern blot analysis or RT–PCR; chromosome 1p36 deletions by *in situ* hybridisation [16]; and CD44 and trk-A expression by immunohistochemistry [17]. The DNA index was measured by flow cytometry where a DNA index of 1.0 denoted diploidy. Histopathology was classified according to Shimada and associates [1]. Patients were followed periodically by physical examination, measurement of tumour markers (urine catecholamines, serum ferritin, NSE and LDH) and radiographically (ultrasonography, CT or magnetic resonance imaging (MRI)).

RESULTS

There were 17 females and 26 males with a mean age of 14 months (range 0.1–72) and median age of 10 months. The mean follow-up was 55 months (range 4–118) and median follow-up was 50 months.

Patient outcome

Forty-two of 43 patients are alive and well (5 with evidence of disease). Surgery was well tolerated except for two major postoperative complications: 1 patient with poor nutritional status at diagnosis developed fungal pneumonia and ileal–ileal intussusception requiring a bowel resection; and a second patient developed significant postlaminectomy scoliosis. Both patients are otherwise alive and well. Twelve patients, including 7 with localised disease and 5 with stage 4S, had recurrent or progressive disease. Progression was defined as any increase in volumetric measurements by radiography or appearance of new disease. Only one had evidence of marrow and distant bony metastases by bone scan. Time to recurrence/progression ranged from 1 to 22 months from diagnosis. Eight of the 12 patients have been followed for at least 15 months (range 15–107) since recurrence/progression. Six of the 12 patients underwent re-operation; only 4 eventually received chemotherapy: one because of marrow and bony metastases 3 months from diagnosis; 1 after recurrence of tumour acquiring 1p deletion; 1 with repeated local recurrence invading vertebral body; and 1 infant with life-threatening enlargement of liver tumours that compromised respiration. The only mortality was due to idiopathic encephalopathy 70 months from diagnosis, presumed to be a paraneoplastic syndrome secondary to neuroblastoma.

Clinical factors of potential prognostic importance

Three patients presented with opsoclonus/myoclonus, 2 with paraplegia from epidural tumours and 2 with renovascular hypertension. Three patients presented with chest masses with airway compromise. Primary sites of origin were adrenal ($n = 14$), pelvis/retroperitoneum ($n = 11$), thoracoabdominal ($n = 2$), mediastinal ($n = 14$) and neck ($n = 2$). Among the 36 patients with localised neuroblastoma, 13 had residual tumour after initial surgery; 2 other patients continued to have elevated urinary VMA excretion for more than 1 year; 19 had local lymph node invasion; and 6 had tumours in the epidural space, 2 of whom were symptomatic with spastic paralysis. Four patients underwent laminectomy and 1 underwent laminoplasty. All recovered from surgery and none had significant neurological deficits. Three patients had local bone and 1 had local marrow invasion. Among the 7 stage 4S patients, all had liver involvement, 3 had marrow disease, 3 had lymph node involvement and 3 had skin tumours. Progressive/recurrent disease occurred in 1 of 3 stage 2A, 2 of 15 stage 2B, 4 of 7 stage 3, and 5 of 7 stage 4S patients (0/11 patients with stage 1 progressed/recurred).

Tumour biological features

The most common adverse biological markers were unfavourable Shimada histopathology (9/40 evaluable tumours) and diploidy (7/39 tumours tested); only 3 tumours had both and all 3 patients had disease progression. One stage 1 and one stage 2B completely resected tumours had *MYCN* amplification, the latter with 1p-LOH. Although both patients are alive and well, without cytotoxic therapy, follow-up is short (4 and 10+ months, respectively). One other newly diagnosed tumour had a deletion of the short arm in one of the three chromosome 1 and one patient's recurrent tumour had a deletion of 1p (without *MYCN* amplification) not detected in the primary tumour. Other poor-risk factors were uncommon in this cohort. Tumour trk-A expression was found in 31/33 (one of the 2 negative tumours was also *MYCN* amplified) and CD44 in 33/33 tumours by immunohistochemistry. Both CD44 and trk-A expression remained unchanged in recurrent tumours. A permanent cell line derived from the only one 1p (del)-recurrent tumour was trk-A negative, suggesting that trk-A negativity may have survival

advantage *in vitro*. Although 5 patients had serum ferritin >142 ng/ml, they were all within normal limits when corrected for age and assay method. None of the patients had LDH >1500 U/l.

DISCUSSION

Patients with local-regional/4S neuroblastoma can survive without cytotoxic therapy. Over a 10-year period at Memorial Sloan-Kettering Cancer Center, 43 consecutive, non-stage 4 neuroblastoma patients were consistently treated initially with surgery and observation. Except for one death from paraneoplastic encephalopathy, no patient died of disease. This excellent survival occurred despite the presence of adverse clinical, pathological and biological factors. The only child who developed distant bony disease 3.4 months after diagnosis had a combination of risk factors: age > 3 years, diploidy, lymph node invasion and unfavourable histology. Despite 12 recurrences/progressions, necessitating re-operation in 9 and chemotherapy in 4, 42/43 patients are alive and well.

This cohort of 43 patients was initially staged using INSS [13, 14]. Our results clearly suggest that clinical staging has powerful therapeutic implications, over and above many prognostic factors known to date. While CD44 and trk-a protein expression appears homogeneous among such tumours, both are expressed in approximately 25–33% of stage 4 metastatic neuroblastomas [18]. The power of the Evans/INSS staging system lies in the distinction of stage 4 by virtue of distant bony metastasis. Our results suggest that localised/regional/4S tumours identified by clinical staging are highly correlated with a benign biology which remains invariant irrespective of when (diagnosis or recurrence) and where (primary site or lymph nodes/liver/marrow) they are sampled. In the absence of cytotoxic therapy, there is no biological force driving these tumours to progress to malignant stage 4 disease. This clearly contrasts with stage 4 neuroblastoma where widespread disease to bone (+/− bone marrow) is the hallmark of malignant behaviour. Distant disease in the bone marrow among patients with stage 4S neuroblastoma has traditionally been described as metastatic, implying their descendence and/or malignant transformation from the primary tumour lineage. To explain spontaneous regression, some have postulated unique cell death programmes among these 'malignant lineages' or host anti-tumour mechanisms, although no evidence exists. Alternatively, others have proposed multiclonality to explain sites of distant disease as multiple clones of benign neuroblastoma [19]. Novel molecular tools of clonality analysis may be necessary to resolve this puzzle [20]. If the conventional paradigm of tumour progression from primary to metastatic sites is characteristic of neuroblastoma, it is surprising that we have not seen more clinical examples supporting this dogma among our patients over a 10-year period. It appears that stage 4 tumour generally metastasises at the time of inception, the process of malignant transformation being tightly linked to that of distant invasion.

We analysed the clinical/pathological and biological factors that may predict disease recurrence/progression. It appears that unresectable tumour and clinical stage correlate strongly with tumour recurrence/progression, the probability of which decreases from 4S > stage 3 > stage 2 > stage 1. Following primary surgery at diagnosis, 4 of the 7 stage 3 patients had gross unresectable tumours and all 4 tumours enlarged, 2 of which required further surgery. *MYC*N amplification has always been thought to herald an aggressive clinical behaviour for localised/stage 4S patients [3, 21, 22]. However, two recent reports of patients with *MYC*N amplified stage I/II neuroblastomas suggested that prolonged survival without chemoradiotherapy was possible [23, 24]. The 2 patients in our series with *MYC*N amplification were only recently diagnosed, and a much longer follow-up is needed. Similarly, since only two tumours had 1p deletion, the prognostic import of this chromosomal abnormality in localised/4S patients with completely resectable tumours remains to be determined in a much larger series.

The excellent outcome (42/43) despite recurrence highlights the importance of using survival as endpoints for non-stage 4 neuroblastoma, where progression-free survival as a surrogate measure of outcome does not reflect the true biological implication of this disease. Twenty-eight per cent (12 patients) recurrence/progression rate is comparable to previous reports, which described patients treated with varying amounts of cytotoxic therapy [25]. Of the 12 patients, only 4 were subsequently treated with chemotherapy. However, unlike previous studies, the survival rate after recurrence in our series is clearly superior. Obviously, longer follow-up is necessary, since 6 patients in our series are only 4–12 months from diagnosis and further tumour progression is possible. Nevertheless, it is reassuring that all 12 patients with recurrence/progression have remained clinically well. It is possible that in addition to acute toxic side-effects with potential lethal complications, some cytotoxic therapy, such as alkylators and etoposide, may accelerate the malignant transformation process.

Cytotoxic therapy is not traditionally used for patients whose tumours have no regional or distant spread (stage 1). However, limited treatment is widely accepted for stage 4S, although in no published series has a majority of such patients been managed consistently with surgery or observation alone. Chemoradiotherapy has also been advocated for those patients with (a) residual disease postsurgery, (b) lymph node involvement or (c) local or distant tumour recurrence. Our observations should raise doubts about current widely adopted cytotoxic treatment regimens for non-stage 4 tumours. Exceptions might include patients with large liver tumours causing inoperable mechanical problems or those with several adverse biological features [22]. Since at least one third of neuroblastoma present with local-regional/4S neuroblastoma, significant morbidity can be spared and health costs minimised if our observations are confirmed in a larger series. Although a comprehensive staging evaluation in conjunction with an analysis of biological markers is essential, this cost is dwarfed by that of chemoradiotherapy, their acute sequelae and their long-term economic and health effects [26]. Although decades of long-term follow-up will be necessary before one is certain that all of these tumours will not continue to recur, the overall favourable outcome of these 43 patients with non-stage 4 neuroblastoma has compelled us to rethink our treatment strategy and to revisit the paradigm underlying our approach to this disease.

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