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ORIGINAL REPORT

Prediction of *MYCN* Amplification in Neuroblastoma Using Serum DNA and Real-Time Quantitative Polymerase Chain Reaction

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A B S T R A C T

Purpose

MYCN amplification (MNA) indicates a poor prognosis in neuroblastoma (NB) and is routinely assayed for therapy stratification. We aimed to develop a diagnostic tool to predict *MYCN* status using serum DNA, which, in cancer patients, predominantly originates from tumor-released DNA.

Patients and Methods

Using DNA-based real-time quantitative polymerase chain reaction, we simultaneously quantified *MYCN* (2p24) and a reference gene, *NAGK* (2p12), and evaluated *MYCN* copy number as an *MYCN/NAGK* (*M/N*) ratio in 87 NB patients whose *MYCN* status had been determined by Southern blotting. Of these patients, 17 had *MYCN*-amplified NB, and 70 had nonamplified NB.

Results

The serum *M/N* ratio in the MNA group (median, 199.32; range, 17.1 to 901.6; 99% CI, 107.0 to 528.7) was significantly ($P < .001$) higher than the ratio in the non-MNA group (median, 0.87; range, 0.25 to 4.6; 99% CI, 0.82 to 1.26; Mann-Whitney *U* test). The sensitivity and specificity of the serum *M/N* ratio as a diagnostic test were both 100% when the serum *M/N* ratio cutoff was set at 10.0. Among six MNA patients whose clinical courses were followed, the serum ratios decreased to the normal range in the patients in remission (n = 3), whereas the ratios increased to high levels in the patients who relapsed (n = 2) or failed to achieve remission (n = 1).

Conclusion

Measurement of the serum *M/N* ratio seems to be a promising method for accurately assessing *MYCN* status in NB, although a larger set of patients needs to be examined to confirm this result.

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Neuroblastoma (NB) is the most common extracranial solid tumor in children and is characterized by a wide range of clinical behaviors, from spontaneous regression to rapid progression with a fatal outcome. The clinical heterogeneity has been reported to be associated with a variety of biologic features of NB. One such aberration, *MYCN* amplification (MNA; ie, creation of multiple copies of the *MYCN* gene in the nuclei of tumor cells), is strongly associated with rapid tumor progression and a poor outcome. MNA is detected in 4% of patients in the early stages of NB, 8% of patients in stage 4S, and approximately 30% of patients in advanced stages. Currently, assessment of *MYCN* status is essential for determining therapy stratification in NB.[1-6] Having rapid access to selected biologic data for each tumor has become increasingly important in

routing patients to appropriate therapies. Several years ago, fluorescence in situ hybridization (FISH) replaced Southern blotting as the most accurate and timely way of evaluating tumors for MNA. Using FISH, the turnaround time for results was shortened from weeks to days, making its use in clinical trials realistic.

In this study, we describe a real-time polymerase chain reaction (PCR) method for evaluating *MYCN* status that shortens the turnaround for results to just a few hours. Furthermore, to facilitate the evaluation of *MYCN* status of tumors, we used serum DNA for the PCR template, which, in cancer patients, predominantly consists of tumor-released DNA.[7] Quantification of serum DNA has also been proposed as a screening tool for early detection of lung cancer.[8] Several groups were able to detect tumor-related aberrations, such as loss of heterozygosity and mutations in the *p53* gene, using the serum DNA of patients with a malignant tumor.[9-11]

Recently, Combaret et al[12] reported that high levels of *MYCN* DNA were present in the peripheral blood of patients with *MYCN*-amplified NB. However, they evaluated serum *MYCN* (2p24) dosage based on PCR without a reference gene, so their assay could be influenced by the quality of the template DNA or a numerical change of chromosome 2. To avoid these problems, we used DNA-based real-time quantitative PCR and a single copy reference gene, the *N-acetylglucosamine kinase* gene (*NAGK*; 2p12), so that *MYCN* copy number per chromosome 2 could be evaluated as the *MYCN/NAGK* (*M/N*) ratio. *NAGK* was chosen because it is on the same chromosome as *MYCN* but sufficiently distant from the region spanned by the *MYCN* amplicon (2p12 *v* 2p24)[13] that a numerical change in chromosome 2 would not affect the *M/N* ratio. The diagnostic performance of the test was evaluated in patients with an NB whose *MYCN* status had been determined by Southern blotting.

Subjects

Eighty-seven patients diagnosed with NB at the Hospital of the Kyoto Prefectural University of Medicine and Chiba Cancer Center Research Institute were enrolled onto this study with the informed consent of their parents. The studies were conducted under research protocols approved by each institutional review board. At the time of diagnosis, 44 patients were younger than 1 year, and 43 were between 1 and 13 years of age. Seventeen of the patients had MNA, and 70 patients did not have MNA, as determined by Southern blotting. According to the International Neuroblastoma Staging System,[4] the 17 children with MNA included one patient each in stage 1 and 2B, two in stage 3, and 13 in stage 4, whereas the 70 children without MNA included 22 in stage 1, 18 in stage 2A and 2B, five in stage 4S, seven in stage 3, and 18 in stage 4.

Twelve of the 17 patients with MNA and 33 of the 70 nonamplified patients were also analyzed by dual-color FISH technique, as previously described,[14] using an *MYCN* probe (pNb101) and a chromosome 2 centromere probe (D2Z). FISH results of these patients were consistent with the Southern blotting results, although three of the patients who were diagnosed as non-MNA by Southern blotting were found to have one to four extra copies of the *MYCN* gene relative to the chromosome 2 centromere number by FISH. This low level of amplification has been defined as *MYCN* gain, which is an intermediate stage between MNA and non-MNA.[15] Because the prognostic significance of *MYCN* gain is still unclear, these patients were classified as non-MNA according to the Southern blotting results.

Sample Preparation

Tumor specimens were surgically resected and immediately stored at −80°C. Peripheral blood was obtained from each patient before any therapy and surgery. To avoid contamination of serum DNA by the DNA from WBCs, we prepared serum exclusively from the liquid fraction of clotted blood after centrifugation at 1,000 × *g* for 10 minutes and stored it at −20°C until DNA extraction.

DNA Isolation

DNA was extracted from tissues and serum samples by using the QIAmp tissue and blood kits (Qiagen, GmbH, Hilden, Germany), respectively, according to the manufacturer's protocols. For each patient, 200 μL of the stored serum was used for extraction of free DNA.

Real-Time Quantitative PCR

TaqMan PCR was performed using the ABI Prism 5700 Sequence Detection System (Applied Biosystems, Foster City, CA). The PCR mixture contained TaqMan universal PCR master mix (Applied Biosystems), 200 nmol/L of each primer, and 100 nmol/L of fluorogenic probe. The principle of the TaqMan analysis has been described previously in detail.[16-18] In addition to the *MYCN* sequence, *NAGK* (GenBank accession No. NM 017567) located at 2p12 was simultaneously measured as a single-copy reference gene. The sequence of primers and the TaqMan probe used for *MYCN* and *NAGK* are as follows: *MYCN* forward, 5′-GTGCTCTCCAATTCTCGCCT-3′; *MYCN* reverse, 5′-GATGGCCTAGAGGAGGGCT-3′; *MYCN* probe, 5′-FAM-CACTAAAGTTCCTTCCACCCTCTCCT-TAMRA-3′; *NAGK* forward, 5′-TGGGCAGACACATCGTAGCA-3′; *NAGK* reverse, 5′-CACCTTCACTCCCACCTCAAC-3′; and *NAGK* probe, 5′-VIC-TGTTGCCCGAGATTGACCCGGT-TAMRA-3′. All PCR reactions were performed with one cycle of 95°C for 5 minutes, followed by PCR amplification with 50 cycles of 95°C for 15 seconds and 60°C for 1 minute. Standard curves were constructed in each PCR run with four-fold serial dilutions containing 20, 5, 1.25, 0.3125, and 0.078125 ng/μL of a healthy donor's DNA in addition to 20 ng/μL of salmon sperm DNA, and the dosages of the target genes in each sample were interpolated using these standard curves. The *MYCN* copy number of a sample of DNA was determined by the ratio of the *MYCN* dosage to the *NAGK* dosage (*M/N* ratio). Copy numbers were expressed as the average of two measurements.

Effect of WBC Contamination

To assess the effect of WBC contamination in serum samples on the serum *M/N* ratio, we measured the serum *M/N* ratio using DNA extracted from a series of WBC-contaminated serum samples. The samples were prepared by adding 0, 1×10, 1×10^2, 1×10^3, 1×10^4, and 1×10^5 of WBCs from a healthy donor to 200 μL of serum from a *MYCN*-amplified patient.

Statistical Methods

The difference in the serum *M/N* ratio between the MNA and non-MNA groups was assessed using the Mann-Whitney *U* test. $P < .05$ was judged as significant.

Serum M/N *Ratio As a Predictor of* MYCN *Status of Tumor*

Serum *M/N* ratios could be determined in approximately 4 hours by real-time quantitative PCR. Figure 1 shows the distribution of the serum *M/N* ratio in the MNA and non-MNA groups at the time of diagnosis. The serum *M/N* ratio in the MNA group (n = 17; median, 199.32; range, 17.1 to 901.6; 99% CI, 107.0 to 528.7) was significantly ($P < .001$) higher than the ratio in the non-MNA group (n = 70; median, 0.87; range, 0.25 to 4.6; 99% CI, 0.82 to 1.26). In fact, there was no overlap between the two groups in the limited number of patients examined in this study. As a cutoff for the serum *M/N* ratio to distinguish between MNA and non-MNA patients, we empirically chose a value of 10, which was in the middle of the two ranges. With this value, the sensitivity and specificity of the serum *M/N* ratio as a diagnostic test to distinguish patients with MNA from those without MNA were both 100% for our limited number of patients. That is, the serum *M/N* ratio was in complete agreement with the Southern blotting results. The positive and negative predictive values were 100%. The serum *M/N* ratios were also consistent with results obtained by FISH for 45 of the patients (FISH analyses were performed in 12 of the 17 MNA patients and in 33 of the 70 nonamplified patients). Three of the patients who had one to four extra copies of the *MYCN* gene relative to chromosome 2 centromere number, as determined by FISH, also had slightly elevated serum *M/N* ratios (2.5, 3.3, and 4.6).

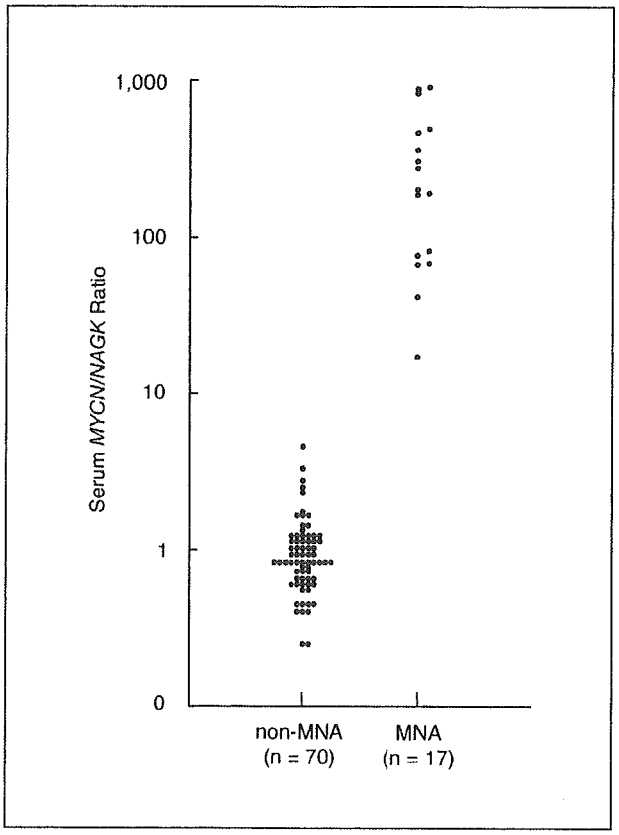

Fig 1. A scatter plot of serum *MYCN/NAGK* ratio in patients with *MYCN*-amplified (MNA) and nonamplified (non MNA) neuroblastoma. The serum *MYCN/NAGK* ratio was significantly ($P < .001$) higher in the MNA group (median, 199.32; range, 17.1 to 901.6; 99% CI, 107.0 to 528.7) than in the non MNA group (median, 0.87; range, 0.25 to 4.6; 99% CI, 0.82 to 1.26, Mann Whitney *U* test).

Change in Serum M/N *Ratio Levels During Follow-Up*

To evaluate whether an increase in the serum *M/N* ratio can be used as an indicator of relapse, we measured serum *M/N* ratios at several points in the clinical courses of six patients with MNA (Fig 2). In three patients who were in complete remission (patients 1, 2, and 3), the serum *M/N* ratios decreased to the normal range and were consistently low. In contrast, in one patient who failed to achieve remission (patient 4), the serum *M/N* ratio did not decrease to the normal range and remained at a high level until his death. In the other patients who experienced recurrence after remission (patients 5 and 6), the serum *M/N* ratio first decreased to the normal range and then increased beyond the cutoff value by the time of diagnosis.

Effect of WBC Contamination on Serum M/N *Ratio*

We found that a high serum *M/N* ratio could be masked by the presence of WBC. The *M/N* ratio of serum from an *MYCN*-amplified patient decreased with increasing WBC contamination (Fig 3). When 200 μL of serum was contaminated with 1×10^5 of WBC, corresponding to approximately one fortieth of the WBC concentration in normal whole blood, the serum *M/N* ratio decreased below the cutoff level.

Serum markers, such as ferritin,[19] lactic dehydrogenase,[20] and neuron-specific enolase,[21] have been proposed as prognostic markers of NB, although they have shown little prognostic value. Recently, elevated levels of plasma midkine have been reported to correlate with a poor prognosis. However, the significance of this finding is controversial because plasma midkine levels are highest in stage 4S patients.[22] Therefore, a noninvasive assay of tumor-related

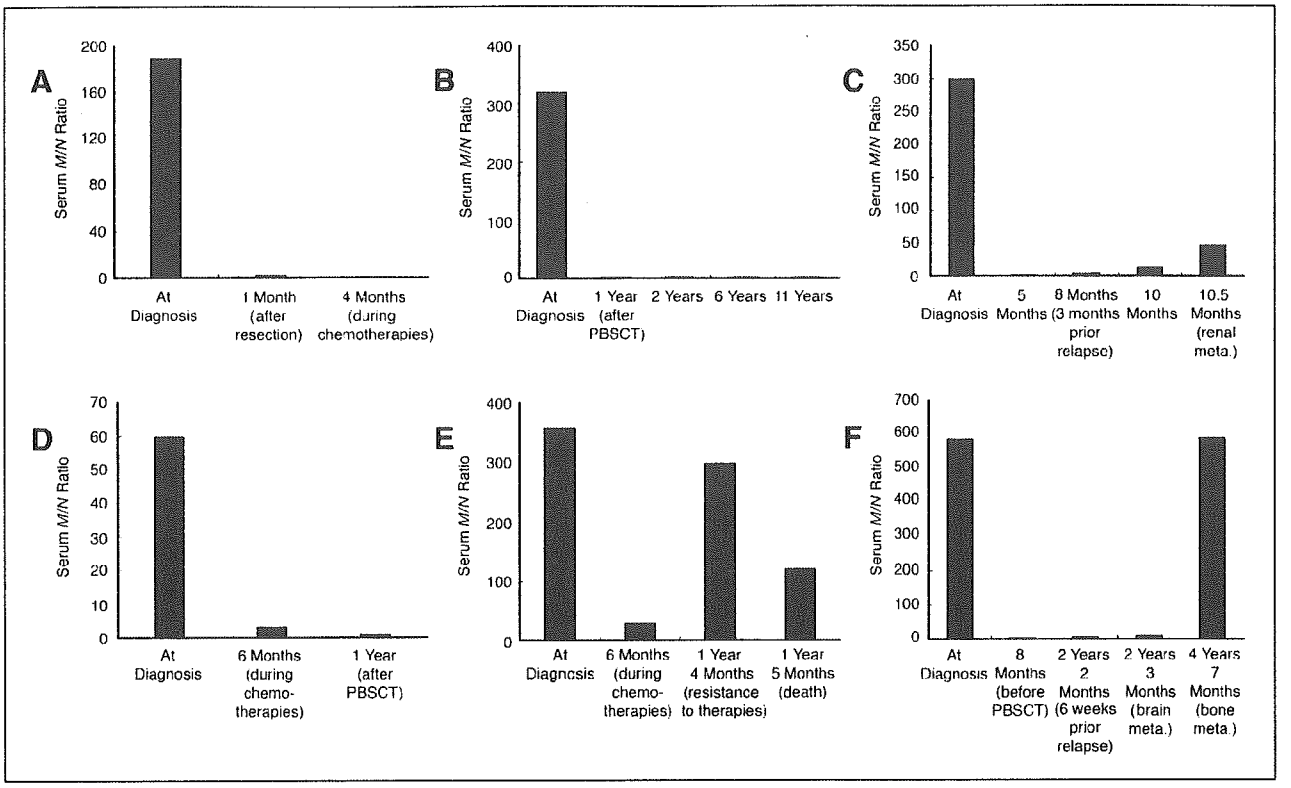

Fig 2. Changes in serum *MYCN/NAGK* (*M/N*) ratio levels of six patients with *MYCN* amplification during follow-up. PBSCT, peripheral-blood stem-cell transfusion; meta., metastasis. (A) Patient 1; (B) patient 3; (C) patient 5; (D) patient 2; (E) patient 4; (F) patient 6.

genetic aberrations using serum DNA is desirable for the assessment of prognosis and therapy stratification at the time of diagnosis. Among the tumor-related genetic aberrations detected in NB, MNA was of greatest interest to us because of its significant prognostic value.

By using DNA-based real-time quantitative PCR with a single-copy reference gene, we have demonstrated that the *M/N* ratio in serum DNA is a valuable diagnostic tool to discriminate MNA patients from non-MNA patients. The serum *M/N* ratio in the MNA group was significantly higher than the ratio in the non-MNA group, without an overlap. The highest sensitivity (100%), highest specificity (100%), highest positive predictive value (100%), and highest negative predictive value (100%) were obtained with a serum *M/N* ratio cutoff value of 10.0. Furthermore, we found an elevated level of the serum *M/N* ratio in a stage 1 patient and a stage 2B patient with MNA (188.7 and 901.6, respectively), even though the tumor was localized in these patients. This suggests that tumors could release a significant amount of genomic DNA into the systemic circulation even at an early stage. Furthermore, Sozzi et al[23] reported that the concentration of plasma DNA in 84 lung cancer patients was higher than the concentration in 43 controls, regardless of the tumor stage, and suggested that circulating DNA in peripheral blood was an early event in lung carcinogenesis.

Another clinical benefit of the serum *M/N* assay is that it could be used as a marker to monitor therapeutic efficacy and recurrence after therapies. The serum *M/N* ratio decreased to the normal range in the patients in remission but remained at a high level in the patient who failed to achieve remission. Furthermore, in two patients with recurrence after remission, the serum *M/N* ratio initially decreased to the normal range but then increased beyond the cutoff value by the time of diagnosis. The serum *M/N* ratio did not increase to the initial level as long as the metastasis was localized in the brain, but it did increase to the initial level when the patient later developed a bone metastasis (patient 6). This is noteworthy because it suggests that a brain metastasis releases genomic DNA into the systemic circulation less easily than extracranial tumors. If this is confirmed by examination of additional patients, then it is possible that tumors localized in brain could be overlooked with diagnostic assays based on serum DNA.

A possible pitfall of our serum *M/N* assay is that a high serum *M/N* ratio could be reduced by WBC contamination (Fig 3). This could be a result of dilution of tumor DNA with the WBC DNA, which would be expected to have an *M/N* ratio of 1. Therefore, the importance of removing WBCs from serum should be addressed in diagnostic assays

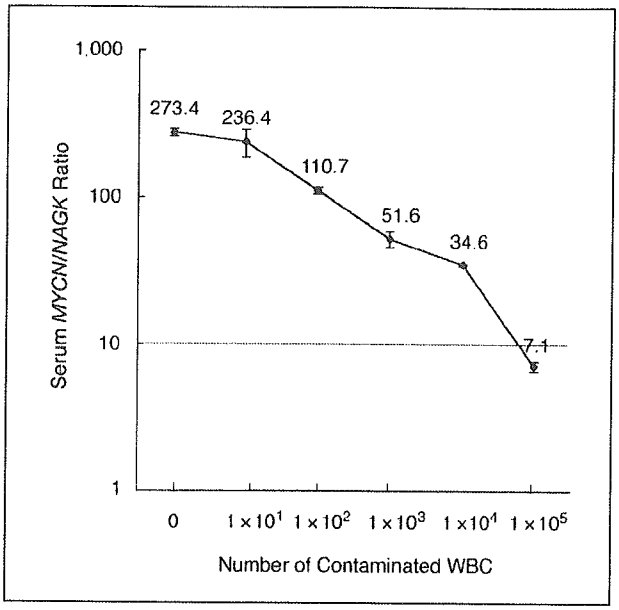

Fig 3. Influence of WBC contamination in serum samples on the serum *MYCN/NAGK* ratio. Data are presented as the mean ± standard deviation of duplicate measurements. The transverse line represents a *MYCN/NAGK* ratio cutoff value of 10.0

that use serum DNA. For the same reason, a predominance of any nontumor DNA in serum may lower an elevated *M/N* ratio of an *MYCN*-amplified patient. However, this assay can be accurate on the premise that, in cancer patients, serum DNA predominantly consists of tumor-released DNA.[7] In addition, the use of serum DNA as a diagnostic tool in lung cancer patients has resulted in a diversity of findings, suggesting that these differences likely reflect variations in the manner in which the blood specimens were collected and handled and variations in the methods by which the assay were conducted.[24] Therefore, it is necessary to standardize the serum collection procedure to ensure that different laboratories obtain the same result with a given blood sample. An additional high-speed centrifugation step (16,000 × *g* for 5 minutes) was found to eliminate cellular contamination even after thawing of stored samples.[25] By using the appropriate centrifugation methods, we believe that WBC-free serum can be reliably achieved.

Although a large set of patients needs to be studied to verify the accuracy of this assay and to set an appropriate cutoff, our results are promising and need to be further tested. The advantages of this method are that it takes only 4 hours and much less effort than FISH and Southern blotting, which should make this assay an alternative to these other methods for determining *MYCN* status. A third advantage is that the serum *M/N* ratio seems to be a promising indicator of therapeutic efficacy and relapse in the follow-up of patients with MNA, although more patients need to be examined to confirm its reliability.

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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Epidural compression in neuroblastoma: Diagnostic and therapeutic aspects

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Abstract

The involvement by tumour of intervertebral foramina and the consequent invasion of the spinal space, accompanied or not by neurological symptoms, represent a well-recognised pattern of presentation of neuroblastoma. The main peculiarity of this condition stands in the fact that, in case of its late detection or inadequate treatment, severe, permanent neurological compromise may ensue. Surprisingly enough, remarkable disagreements still exist regarding its optimal treatment and the related literature provide contrasting indications at this respect. The neurosurgical and the chemotherapeutic approaches have equally convinced supporters, while the use of radiation therapy is uncommon, possibly without good reasons. This mini-review intends to report

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the clinical experiences of the major Paediatric Oncology Groups with the aim to collect as many data as possible in the perspective of establishing common guidelines for proper diagnosing and treatment of this important complication.

Keywords: Neuroblastoma; Epidural compression; Spinal cord compression; Late effects

1. Introduction (by Audrey E. Evans, Philadelphia)

Signs of cord compression from a paravertebral neuroblastoma make up one of the few emergencies that may occur in paediatric oncology [1–4]. The decision regarding appropriate treatment often depends upon the specialist who first sees the patient: the neurosurgeon or the oncologist. The former knows that decompression can relieve the problem promptly; and it is to hope, is equally aware of the truly major orthopaedic problems with normal growth and development that ensue. The chemotherapist's efforts are usually effective, and no important late complications can be expected from the modest doses of chemotherapy that are usually employed. Days may be needed to relieve the problem, however, is there time? Although not consulted initially, the radiation therapist also knows that irradiation is rapidly beneficial; and with the low doses used to day, has minimal long-term side effects. The choice among these three treatments that are equally effective revolves around the two major issues: time and late effects. The extraordinary resilience of the childhood spinal cord here enters the equation. The dicta concerning irreversible paralysis always supervening after more than a few hours of frank paresis from cord compression do not hold in paediatrics. Full recovery has been observed by me even after several days. Emergency, middle-of-the-night surgery is therefore seldom warranted in this setting and can be planned more deliberately. To be sure, laminectomy uniquely has the advantage of guaranteed immediate relief. It also provides tissue for accurate diagnosis and for various tests and research projects, although these last are a very secondary consideration in this matter. These advantages are offset by the very major scoliotic deformities that ensue. Insofar as radiation therapy is concerned, relatively early responses can be expected without serious adversities later on. A series of treatments over 5 days or so is necessary nonetheless. Moreover, not all institutions have radiotherapy readily available, or the radiotherapy facility may be off-site making daily transport necessary. These several competing aspects were discussed and debated by experts in each of the disciplines involved. Their deliberations are detailed in the report that follows.

2. Diagnostic imaging (by Paolo Tortori-Donati and Andrea Rossi, Genova)

Neuroblastoma invading the intervertebral foramina and the spinal canal typically displays a dumbbell aspect [5]. The intraspinal component usually remains confined extradurally. X-rays may directly show calcified paraspinal masses. More often, indirect signs such as lytic or sclerotic changes in the adjacent bone are seen. Enlargement of one or more neural foramina and widened interpeduncular distances are all also frequently found. MRI adequately depicts the intraspinal extension of the mass. Often, the intraspinal component extends for several metameres both cranially and caudally to the entrance foramen (Fig. 1A and B). Such development is optimally depicted on the coronal plane. The lesion is usually hypointense on T1-weighted images and iso- to hypointense on T2-weighted images due to high cellularity and nuclear-to-cytoplasmatic ratio. However, necrotic–cystic changes, haemorrhages, and calcifications can result in heterogeneous signal behaviour. Contrast enhancement is usually marked. MRI also detects compression and dislocation of the spinal cord. Intramedullary hyperintense signal on T2-weighted images reflects cord oedema in the setting of compression-related myelopathy (Figs. 2 and 3).

Differential diagnosis includes a variety of tumours, such as lymphomas, skeletal and extraosseous Ewing's sarcomas, nephroblastoma and malignant nerve sheath tumours.

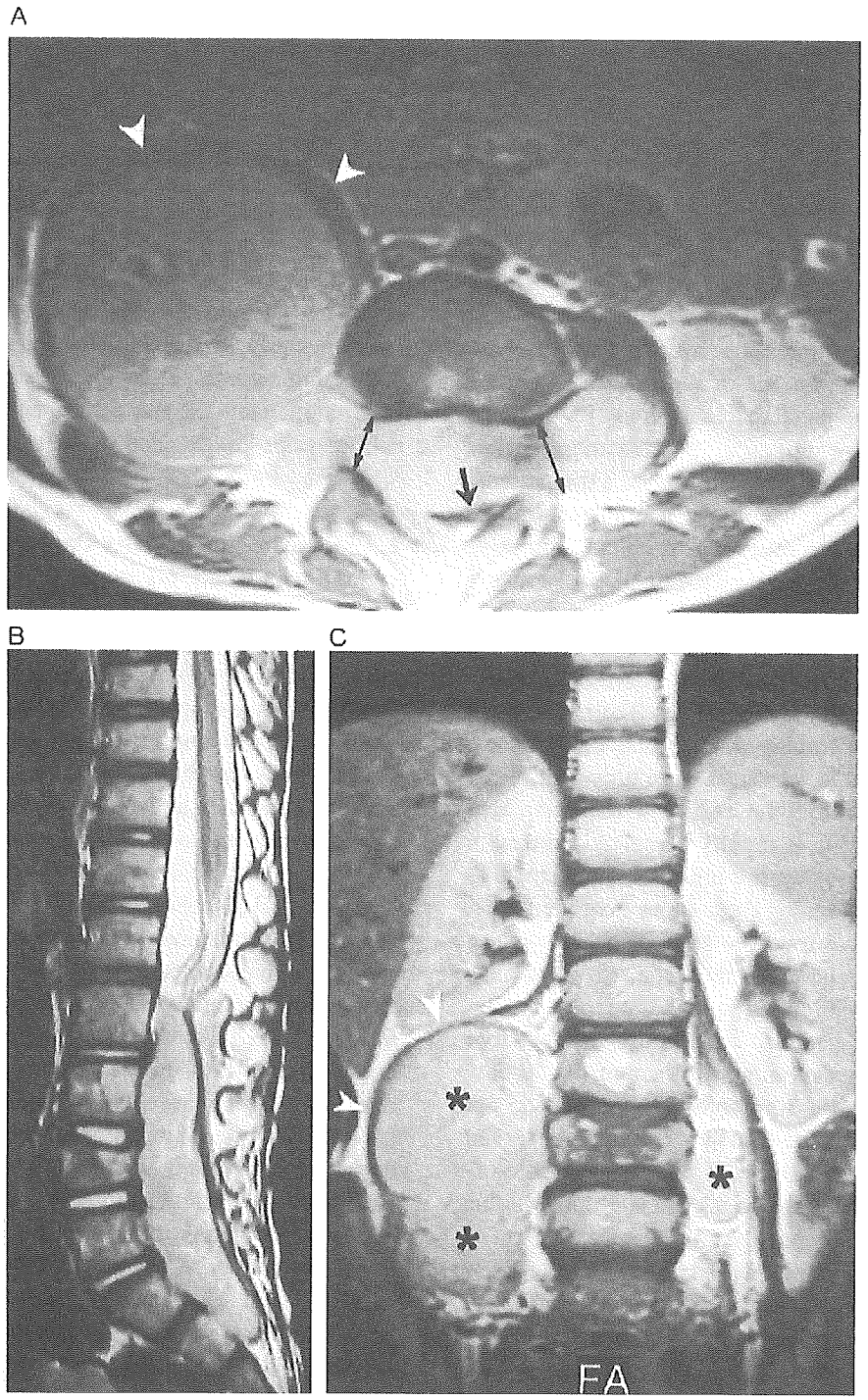

Fig. 1. Retroperitoneal neuroblastoma with bilateral intraspinal extension in a 3-year-old boy. (A) Gd-enhanced coronal T1-weighted image. (B) Sagittal T2-weighted image. (C) Gd-enhanced axial T1-weighted image. Coronal image shows large tumour masses to both sides of the lumbar spine (asterisks, A) and a pathologic L4 vertebral body. The size of the intraspinal extradural component is huge (B). Axial image shows bilateral dumbbell extension through widened neural foramina (double arrows, C) and marked compression of the thecal sac (single arrow, C). Notice marked elevation and displacement of the right psoas muscle (arrowheads, A,C).

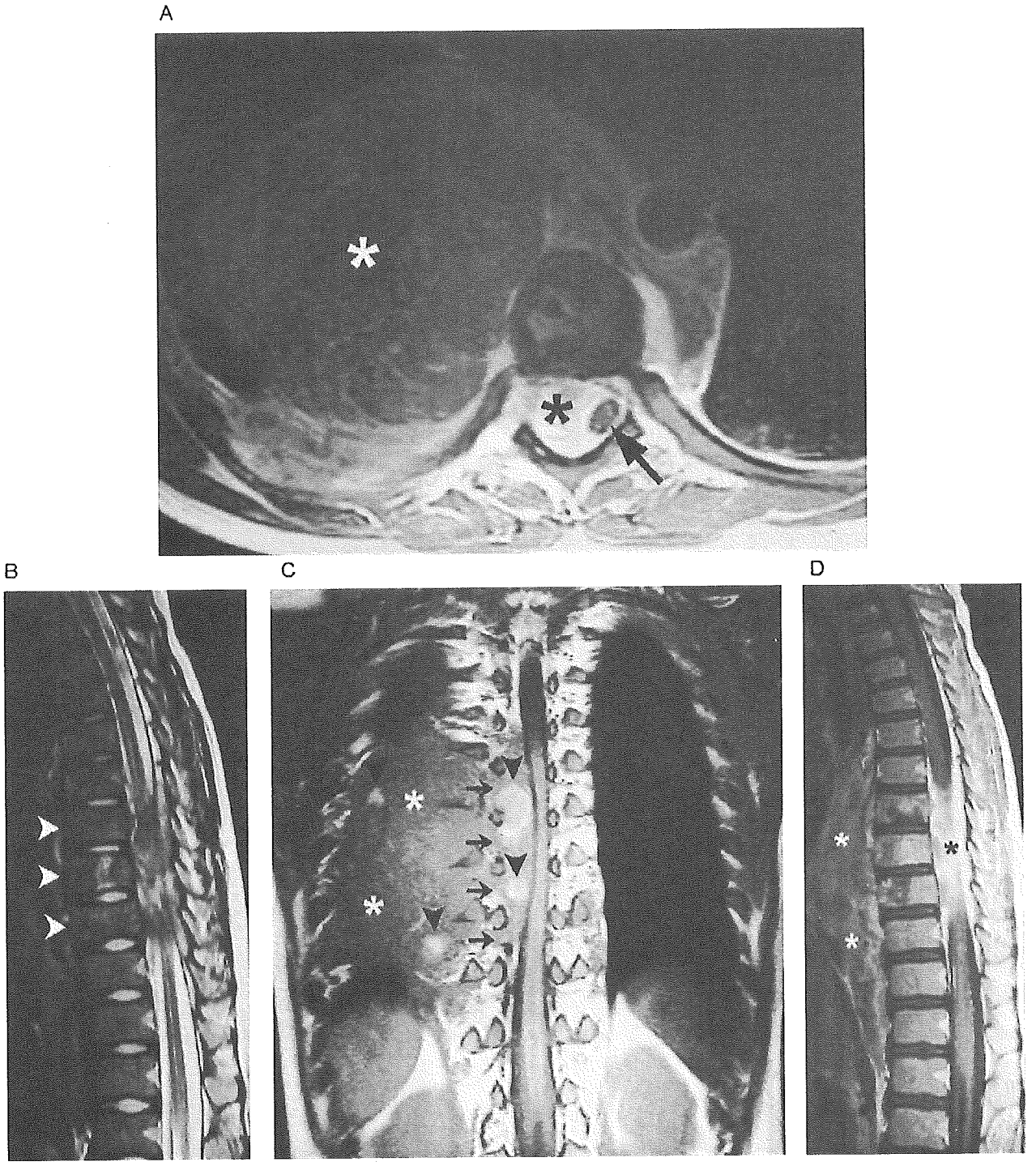

Fig. 2. Thoracic neuroblastoma with intraspinal invasion in a 6-year-old boy. (A) Coronal T1-weighted image. (B) Sagittal T2-weighted image. (C) Gd-enhanced sagittal T1-weighted image. (D) Axial gd-enhanced axial T1-weighted image. Huge thoracic mass (black asterisks, A–D) extends an intraspinal component through four, markedly enlarged neural foramina (arrows, A). This component, hypointense on T2-weighted images (B) and enhancing with contrast material administration (black asterisk, C,D), compresses and engulfs the spinal cord (arrow, D). The mass has numerous hemorrhagic components (arrowheads, A). Three pathologic vertebrae are seen (arrowheads, B).

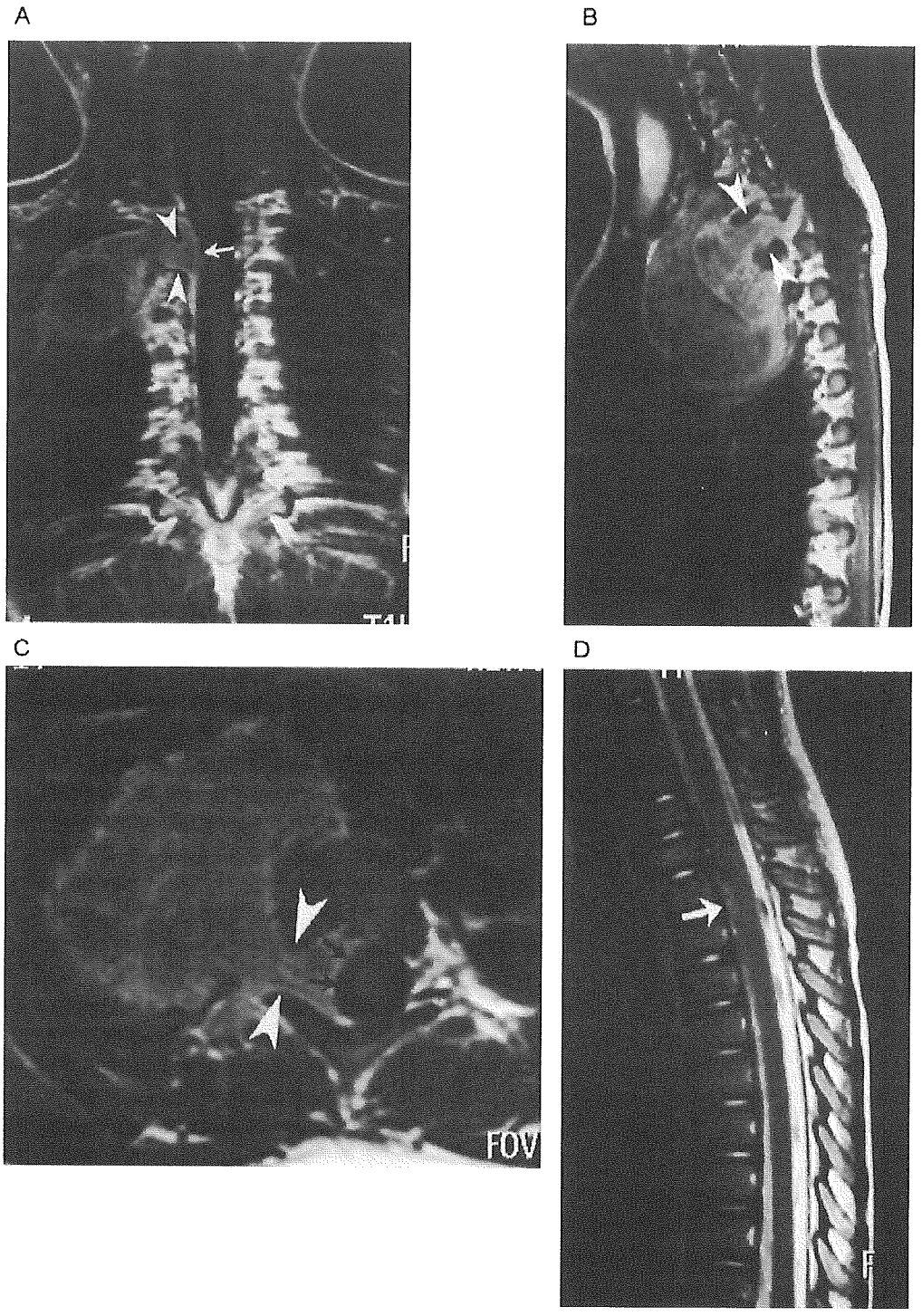

Fig. 3. Thoracic neuroblastoma with intraspinal extension in a 2-year-old girl. (A) Gd-enhanced coronal T1-weighted. (B) Gd-enhanced parasagittal T1-weighted. and (C) Gd-enhanced axial T1-weighted image show thoracic mass with a dumbbell extension through an enlarged neural foramen (arrowheads), invading the epidural space (arrows, A). Notice that, although the thecal sac containing the spinal cord is slightly displaced contralaterally, preservation of the subarachnoid spaces around the cord (arrows, C) indicates absence of spinal cord compression. (D) Midsagittal T2-weighted image shows mild amount of pathological tissue in the ventral epidural space (arrow) and a normal spinal cord.

3. Treatment

3.1. Neurosurgery (by Armando Cama, Genova)

Surgical decompression by laminectomy has been for long time the almost unique treatment modality for epidural compression detected at diagnosis in children with neuroblastoma [6]. Neurosurgeons have always recognised this entity as a true surgical emergency in reason of the peculiar anatomic and vascular features of the spinal cord making it extremely sensitive to any type of injury. Severity of spinal cord damage correlates with site of compression (being more severe at cervical and upper dorsal levels), tumour extension in the spinal canal, and duration of compression. In absence of effective therapy, neurological function tends to deteriorate leading to permanent deficits. In the late 80s, along with the remarkable improvement in diagnostic imaging and the refinement of supportive measures, chemotherapy started to be considered as an alternative therapeutic approach, its main advantage being a lower risk of orthopaedic sequelae. Consequently, the issue of how to treat a child affected by neuroblastoma presenting epidural compression has become matter of strong debate, eventually making it compelling to reach a consensus. At the present, surgical decompression should possibly be considered the preferable option in case of rapidly neurological worsening and of lack of improvement during the administration of chemotherapy. Neurosurgery may also be indicated when the diagnosis of neuroblastoma needs confirmation, as it may occur when catecholamine excretion is normal and bone marrow is free of tumour infiltration.

3.2. Radiation therapy (by Giulio J D'Angio, Philadelphia)

Radiation therapy can be delivered easily and promptly to a small paravertebral tumour with intraspinal extension through the use of a single, posterior field [7]. Larger tumours may require anterior as well as posterior fields to obtain dose homogeneity. A straightforward technique is to deliver 300 cGy to the mass on each of 3 successive days since there is always some urgency, else radiation therapy would not have been elected. The effect on normal growth and development after such a dose given to a field 3–5 vertebral bodies in length is negligible even in babies. Perhaps 3 cm of shortening in total height at maturity can be expected after such irradiation given to an infant. Late effects on the thyroid are the major concern when the neck or upper thoracic spine is treated. This is because even very low scattered doses of radiation can cause trouble with function, and/or give rise to growths, both benign and malignant. More modern techniques are available that localize the radiation therapy very precisely to the tumour volume. Adjoining tissues and structures receive only a fraction of the tumour dose, but these methods require more elaborate planning that may take days. The treatment time is also longer; that is, the time the child must lie still on the table. This is essential if the full advantages of these methods are to be enjoyed. Anaesthesia may then be necessary in children who cannot cooperate because of young age or temperament. The problems with post-irradiation thyroid difficulties after upper spine radiation therapy also are not obviated, because the gland receives scattered radiation even under the most stringent limitations of field edges. On balance, the simpler field arrangements are probably the best given the setting in which radiation therapy is to be employed. They are quick, and easy to do.

3.3. Chemotherapy (by Bruno De Bernardi, Genova)

The effectiveness of first line chemotherapy in dumbbell neuroblastoma was first reported by Hayes et al. [8] in 1984. Nine of 11 children with neurological symptoms recovered completely and two remained paraplegic. In 1989, Sanderson et al. [9] reported two children with paraplegia and two with paraparesis, who fully recovered being treated with OPEC Regimen. Based on these favourable results more and more children with epidural neuroblastoma have subsequently been spared a neurosurgical intervention. However, the identification of which children can be safely treated by chemotherapy alone has not been clarified yet although in this mini-review two series report on children treated prevalently or exclusively by chemotherapy.

4. Clinical experiences

4.1. Italy (Maria Luisa Garré, Genova)

The Italian Cooperative Group for Neuroblastoma published in 2001 a series of 76 cases of symptomatic epidural compression that occurred in 1462 children with neuroblastoma recruited between 1979 and 1998 [10]. Chemotherapy and decompressive laminectomy had comparable ability to relieve neurological symptoms, although none of the children presenting with paraplegia recovered.

Based on this retrospective study, children with neuroblastoma presenting with symptomatic epidural compression in the subsequent period 1999–2003 were preferentially treated with chemotherapy and followed prospectively. They are the object of the present report.

4.1.1. Patients and methods

Children with previously untreated neuroblastoma and symptomatic epidural compression at diagnosis were included in this analysis. Data concerning symptoms and neurological status at presentation, time elapsed between first symptoms and diagnosis, initial therapeutic approach, and neurological outcome were registered. Motor impairment was scored as follows: *grade 1*, mild hypostenia with walking disability for legs, poor difficulty in raising hands above head for arms; *grade 2*, moderate hypostenia with inability to walk and make movements against gravity, or raise hands above head; *grade 3*, severe hypostenia with paraplegia, no elicitable tendon reflexes or muscular movements.

4.1.2. Results

Of a total of 530 children with neuroblastoma 26 (4.9%) presented with symptomatic epidural compression. Eleven were male and 15 female. Age ranged form 0 to 18 years (median, 14 months). Follow-up ranged from 12 to 55 months (median, 22). Patient demographics, neurological presentation and treatment are shown in Table 1 and look comparable to the previously published series, but for the severity of motor impairment at diagnosis.

Five patients presented with motor deficit of grade 1, 16 of grade 2 and five of grade 3. Ten children complained of back pain, 10 had sphincteric and five had sensitivity dysfunction. Eight out of 26 children epidural compression was treated by laminotomy. Three of them did not receive additional treatment, and five received chemotherapy according to disease stage. In the remaining 18 children, the initial treatment was chemotherapy. Two of them, who did not respond, underwent laminotomy with symptomatic benefit. One more patient, who had a partial response to chemotherapy, underwent delayed laminectomy to remove residual intraspinal disease and obtained further neurological improvement. No patients was treated with radiation therapy as first or secondary choice.

Twenty-two out of the 26 cases (85%) are alive with a follow-up of 12–55 months (median, 22). The four remaining children died of tumour progression. Neurological response in relation to disease stage and initial treatment are reported in Table 2.

Table 1
Patient characteristics and clinical presentation

Characteristics	Present series		Retrospective study
	N	%	%
Sex			
M/F	11/15	42/58	50/50
Age			
<12 months/≥12 months	12/14	46/54	40/60
Primary tumour site			
Thorax	12	46	37
Abdomen	12	46	55
Pelvis	1	4	7
Other	1	4	1
Stage			
2/3	20	77	71
4/4s	6	23	29
Neurological abnormalities			
Motor deficit grade 1	5	19	57
Motor deficit grade 2	16	61	29
Motor deficit grade 3	5	19	13
Time between neurological symptoms and diagnosis			
<1 week/1–4 weeks	5/10	19/38	17/37
1–2 months/>2 months	3/6	11/23	18/28
Not evaluable	2	8	0
Specific therapy			
Surgery	8	35	31
Chemotherapy	18	65	58
Radiotherapy	0	0	12
Secondary surgery	2	8	0

Table 2
Neurological response in relation to therapy and disease extent

Therapy	Total	Neurological response					
		Complete		Partial		No	
		Localised	Stage 4	Localised	Stage 4	Localised	Stage 4
Chemotherapy	18	7	–	6	2	2	1
Laminotomy	8	1	–	2	2	2	1
Total	26	8	–	8	4	4	2

4.1.3. Long-term sequelae

In our previous study, children treated with chemotherapy only had less orthopaedic sequelae compared to those treated by surgery or radiation therapy. In the present series, out of 14 children with follow-up above 2 years, seven have developed orthopaedic sequelae, including a single instance of scoliosis following laminotomy. More details on these cases are reported later on.

4.1.4. Conclusions

The percentage of children with neuroblastoma and epidural compression in this series is 4.9% (5.2% in the previous publication). In analogy with our previous report, about 50% of patients were younger than one year, and the proportion of thoracic and of localised tumours was higher than in the remaining population. No variation in the time elapsed from onset of symptoms to diagnosis was noted. Our data seem to confirm that chemotherapy is adequate in relieving neurological symptoms in the majority of cases. Neurosurgery was effective in the two cases which did not improve with chemotherapy. Finally, even in the present series a significant number of cases developed neurological and/or orthopaedic sequelae.

4.2. Germany (by Thorsten Simon, Cologne)

4.2.1. Patients and methods

Patients of the Cooperative German trials NB90, NB95 and NB97 with neuroblastoma diagnosed between September 1989 and December 2003, were included in this analysis. For management of symptomatic intraspinal involvement, neurosurgery was strongly recommended. During the last years, the potential of chemotherapy was acknowledged and initial chemotherapy was accepted as an alternative. Radiotherapy was reserved for the management of critically ill patients. Symptomatic transverse myelopathy was defined as presence of paraplegia or impaired motor function of extremities, impaired bladder voiding, or impaired stool control. Complete and incomplete loss of function were distinguished.

4.2.2. Results

The series is made of 1966 patients with median age at diagnosis of 1.3 years (range 0–36). The median observation time was 5.3 years (0.03–16.6). Ninety-three patients (4.7%) presented with clinical symptoms of spinal cord compression at diagnosis. Fourteen of them had paraplegia, and 79 had severely impaired function of legs, bladder and bowel. Children with spinal cord compression were younger than patients without transverse myelopathy (0.73 vs. 1.32 years; $P = .001$), had fewer cases with *MYCN* amplification (6.9 vs. 16.7%; $P = .032$), more localized tumours (74 vs. 52%; $P < .001$), and a far higher proportion with thoracic primary tumour (42% vs. 16%; $P < .001$). The highest frequency of transverse myelopathy was found in stage 3 (Table 3). Details of the presenting symptoms were available in 91/93 patients. Eighty-two presented with motor deficit of the lower extremities, 25 complained of diffuse or localized pain, 13 had bowel incontinence, and 12 bladder dysfunction. The median duration of symptoms prior to diagnosis was 17 days (range 0–495).

Symptomless intraspinal involvement defined as unequivocal presence of tumour tissue in the spinal space without neurological symptoms was found in additional 111 patients. Of note, the proportion of patients with evidence of intraspinal tumour tissue was higher in NB97 (12.9%) compared to NB90 (7.4%), as a consequence of an increased use of MRI instead of CT at diagnosis.

Table 3
Characteristics of 1966 neuroblastoma patients

	Transverse myelopathy		P
	Yes	No	
Patients	93	1873	
Gender			
M/F	45/48	1019/854	
Age			
Mean (years)	.73	1.32	.001
MYCN			
Single copy	67	1287	.032
Amplified	5	259	
Not assessed	21	327	
INSS stage			
1–2–3	69	978	<0.001
4–4s	24	895	
Primary site			
Neck	0	49	<.001
Chest	39	289	
Abdomen	54	1509	
Not detected	0	26	

4.2.3. Treatment

Fifty-two patients (56%) underwent emergency neurosurgery for transverse myelopathy, 41 (44%) received chemotherapy without any preceding surgery. No patient was initially treated with radiotherapy. Thirteen chemotherapy patients underwent neurosurgery after starting chemotherapy. Of 111 patients with asymptomatic intraspinal involvement, 31 underwent neurosurgery.

4.2.4. Outcome

The general 5-year event-free survival was 62±1%, the 5-year overall survival was 72±1%. Compared to other patients, children with initial symptomatic spinal cord compression had a similar 5-year event-free survival (71±1 vs. 61±1%; $P=.6$) but a better 5-year overall survival (85±4 vs. 71±1%; $P=.004$). Separate analysis of localized and stage 4 neuroblastoma echoed these results. In stage 4s no difference was found at all.

Only 1366 patients surviving 2 years or longer were included in the analysis of late effects. Transverse myelopathy was cured without residual symptoms in 47 of 67 patients (Table 4). The duration of symptoms prior to treatment had no impact on the neurological outcome: patients with an history lasting 3 days or less had the same frequency of late myelopathy (3/8 patients) as patients with an history >3 days (17/59; $P=.446$). Increasing the cut-off to 7 days gave similar results ($P=.610$). Patients with complete transverse myelopathy more frequently had residual neurological deficits (6/13=46%) than patients with incomplete transverse myelopathy (14/54=25%). The difference was not significant, possibly because of the small patient numbers ($P=.138$).

There was no clear impact of treatment on long-term neurological outcome: 30 patients started with chemotherapy immediately after the diagnosis was established. Eight of them (27%) presented with residual neurological symptoms during follow-up, compared to 12/37 (32%) who underwent initial neurosurgery ($P=.6$). Among 30 children starting with chemotherapy, 12 had to undergo secondary neurosurgery. The median time interval between the start of chemotherapy and neurosurgery was 122 days (range 2–402). Five of 12 patients (42%) had persisting neurological symptoms, compared to 3/18 (17%) who did not require second line neurosurgery ($P=.14$). The two patients who had early operation performed 2 and 5 days after chemotherapy start had persisting symptoms, whereas 3/10 patients with delayed chemotherapy done 35+ days after chemotherapy start had late neurological impairment.

Scoliosis developed in 39 cases. It was far more common after initial myelopathy (12/67=18%), but it was observed in other patients as well (27/1299=2%; $P<.001$). The proportion of scoliosis was similar among children who underwent neurosurgery for spinal cord compression (8/48=17%), and those who had chemotherapy only (4/19=21%; $P=.46$).

4.2.5. Conclusions

Patients with symptomatic intraspinal involvement by neuroblastoma had a better survival, which might

Table 4
Outcome of 1366 patients surviving >2 years after diagnosis

	Initial transverse myelopathy		P
	Yes	No	
Patients	67	1299	
Residual neurological symptoms			
No	47	1292	<.001
Yes	20	7	
Scoliosis			
No	55	1272	<.001
Yes	12	27	

be explained by their younger age, higher incidence of localized tumours, and lower proportion of *MYCN* amplified tumours. Even when a long time elapsed between first occurrence of symptoms and treatment, complete recovery of neurology was possible and vice versa. The risk of neurological and orthopaedic sequelae appeared to be similar after chemotherapy or neurosurgery as initial treatment. All analyses was limited by the small number of patients.

4.3. Poland (by Walentyna Balwierz, Krakow)

Based on retrospective analysis we present demographics, diagnostic procedures, treatment modalities and results of therapy in patients with neuroblastoma who presented with symptomatic or asymptomatic epidural compression. The observation was finished in December 2003.

4.3.1. Patients demographics

In the years 1997–2003, a total of 185 patients with neuroblastoma were treated in seven centres of the Polish Paediatric Solid Tumours Study Group. Twenty-two patients (12%) had tumour penetrating into the spinal canal at diagnosis.

4.3.2. Symptoms at presentation

Seven/24 patients had no clinical symptoms of epidural compression. Extension of the tumour into the spinal canal was found by radiological imaging in six children and during surgery in one case. In 15 patients (65%), symptoms of epidural compression were present at diagnosis, consisting of paresis in all, sphincteric dysfunction in 10, pain in 4, and sensitivity dysfunction in 2. Most patients had more than one symptom at diagnosis. Paresis with sphincteric dysfunction was the most common presentation. The presence of intraspinal tumour extension was documented by radiological imaging. The median duration of symptoms prior to diagnosis was 1.2 months (range 0.1–4.2).

4.3.3. Treatment

Thirteen children, including nine symptomatic, received chemotherapy as upfront treatment, according to different regimens, depending on patients age and disease extension. Five of these patients had delayed surgery without laminectomy (two with neurological symptoms). Three further patients had delayed laminectomy because of persistent neurological symptoms after chemotherapy. In one of them, infiltration of meninges was found during neurosurgery. In 3/22 children (including two with symptoms), epidural compression was approached by laminectomy as initial treatment. They all received adjuvant chemotherapy according to stage. Six children underwent first upfront surgery on the intrathoracic/abdominal component of the primary tumour and then chemotherapy. None of the patients was treated with radiotherapy as first choice, but three children with symptomatic epidural compression received delayed radiotherapy because of poor response to primary treatment.

4.3.4. Outcome

Fourteen of 22 patients (64%) are alive with no evidence of neuroblastoma, with a follow-up of 5–129 months (median, 45). Two patients are alive with disease. Six children died, including two infants (five from neuroblastoma progression, one from secondary leukaemia). Five-year progression-free survival for infant and non-infant patients is 0.88 and 0.32, respectively. Two of three children with *MYCN* amplification died of disease progression.

4.3.5. Neurological outcome

Five of 15 patients who presented with paresis (including three infants) recovered completely. In eight children (five infants), partial recovery was observed, and in two resolution of symptoms was not obtained.

4.3.6. Complications

No serious complications of laminectomy were observed, except for one case of scoliosis. One asymptomatic child developed Horner's syndrome as a thoracoscopy complication. Leukaemia developed in one infant during the second year of treatment for neuroblastoma.

4.3.7. Conclusions

Due to the small number of patients, variety of treatment modalities employed, and lack of a standardized approach, it is very difficult to evaluate the efficacy of the different therapeutic approaches used. Laminectomy is probably not necessary in most

children with dumbbell neuroblastoma, especially in infants. It is necessary to introduce prospective therapeutic strategies for better disease control. Further improvement of therapeutic strategy for children with neuroblastoma and spinal cord compression calls for association of individual centres in groups which would co-operate in research projects, improving diagnosis and pursuing optimisation of treatment methods.

4.4. United Kingdom (by Joanna Begent, London)

4.4.1. Patients and methods

In order to analyse our experience of neuroblastoma affecting the spinal cord at one UK centre, we conducted a database search followed by retrospective notes analysis. Our aim was to analyse management and outcome of children with stage 2/3 neuroblastoma with spinal involvement treated over a 20-year period, during which 387 children were diagnosed having a neuroblastoma, of whom 115 had stage 2 or 3 disease. We reviewed imaging reports of these 115 children, of whom 22 had evidence of an intraspinal tumour component. Our study looked at 13 girls and 9 boys. The majority of children (72%) presented at <1 year of age (median 0.81, range 0–1.8). Two cases presented at birth. In our cohort, 14 children had stage 3 and 8 had stage 2 disease. Time from first symptoms to presentation varied from 24 h to 3 months (median, 15 days). Eight patients had had over 3 weeks of symptoms prior to presentation. Some of the patients were international transfers. The tumours were spread between sites with nine of the 22 having thoracic and 7/22 having pelvic tumours; the remaining six were lumbar tumours. At presentation all were symptomatic although only 15/22 had neurological symptoms. Eleven children had primarily limb neurology, seven of these had flaccid paralysis; four had bladder/bowel symptoms and seven had non-neurological symptoms—from incidental abdominal masses to a cough. The incidence of sphincteric dysfunction at diagnosis is possibly underestimated, in reason of the fact that most of the patients were infants. None of the patients reported pain or sensitivity disturbances. No child had amplification of the *MYCN* gene in his tumour.

4.4.2. Therapy and results

Treatment strategies varied, although all children had at least two therapeutic modalities. At presentation, three symptomatic children underwent neurosurgery, with partial recovery. Twelve children were treated with chemotherapy first: 9 (75%) had a partial, and 3 (25%) a complete neurological recovery. Five patients also received steroids. In four children, in which chemotherapy did not succeed in relieving neurological symptoms, neurosurgery was performed and it leads to an improvement in three of them (75%). Laminectomy was performed in five cases and laminoplasty in two more recent operations. Fifteen children underwent surgery on the intra thoracic/abdominal component of the primary tumour alone. Twenty-one had chemotherapy from varying protocols (mostly carboplatin/etoposide, or OPEC/OJEC). Two children received radiotherapy as an adjunct to other treatment (16–20 Grey, respectively). There is a wide follow-up interval in our cohort (1–20 years; mean 9.3); all but one of these patients are alive (one patient died from other causes). Overall survival from disease is 100%; however, 50% of children have serious sequelae and only six have none. Six patients cannot walk, all of whom presented with limb neurology. Two are paraplegic, one of whom had a congenital tumour. Five are paraparetic, including the second congenital case. Two of these had laminectomy at diagnosis. Twelve have ongoing urinary incontinence and/or bowel control problems, including the two congenital cases. Ten children have spinal deformity; this group includes 7 of the 15 who did not have spinal surgery and three of the seven who did. Two children have subsequently had corrective surgery (one post-laminectomy). Other problems include two children with significant foot size differences, three with altered sensation in their legs and two suffering with erectile problems. Both children who presented with congenital spinal cord neuroblastoma are severely disabled. All the other non-ambulant children had over 2 weeks of limb neurology prior to presentation. Two of the four were referred from outside the UK. Four of the six children with no long-term sequelae had a history lasting less than a week.

4.4.3. Conclusions

In summary, in our study 19% of children with stage 2/3 neuroblastoma have spinal involvement.

Management of these children has not been consistent, however, prompt treatment clearly improves neurological outcome. Although prognosis from disease is good, long-term morbidity remains high.

4.5. Children oncology group (by Sue Cohn, Howard Katzenstein, and Wendy London; Chicago, Atlanta, and Gainesville, USA)

4.5.1. Patient demographics

Between May 1990 and January 1998, 83 children with intraspinal neuroblastoma were registered on POG NB Biology Protocol #9047 [11]. The age at diagnosis ranged from birth to 13 years and 2 months (median, 10 months). Eight patients had stage A tumours; 23 had stage B disease; 28 had stage C; 21 had stage D; and one patient had stage DS disease. The primary intraspinal component was located in the thoracic region in 55 (66%) of the 83 patients. The estimated survival rate $\pm$ standard error of the entire group at 5 years was $71 \pm 9\%$.

4.5.2. Symptoms at presentation and initial treatment

Forty-three patients had neurological symptoms at diagnosis; 15 patients had severe neurological deficits with paraplegia, five had moderate symptoms consisting of paresis with bowel and/or bladder dysfunction, and 22 had mild deficits with paresis alone. Sixty-six patients received upfront chemotherapy; eight received initial radiotherapy; 23 underwent primary laminectomy; 31 underwent upfront surgical resection without laminectomy, and 25 did not undergo an initial surgical resection.

4.5.3. Neurological outcome

Six of the 15 patients who presented with paralysis completely recovered neurological function; two following surgical decompression with laminectomy, one following chemotherapy, and three following chemotherapy and radiotherapy. In this cohort, the length of time between the development of symptoms and the initiation of therapy was inversely correlated with the degree of neurological recovery. Two of five patients with moderate neurological deficits completely recovered neurological function; one following laminectomy and one following chemotherapy. Seventeen of 22 evaluable children with mild symptoms fully recovered; eight following laminectomy, one following surgical resection without laminectomy and eight following treatment with chemotherapy. No clear correlation between the development of symptoms and the initiation of therapy was seen in the moderately or mildly affected patients. Five of 40 patients who did not have neurological symptoms at the time of diagnosis acquired neurological symptoms following treatment; three developed Horner's syndrome, one parasthesia, and one paralysis following a delayed surgical resection.

4.5.4. Orthopaedic late effects

A total of eight patients (10%) in our series were noted to develop scoliosis. Scoliosis was considered to be severe in one case (curvature $>40°$); three were categorized as moderate curvature (>20 and $<40°$); and four were classified as mild ($<20°$). Seven of the eight patients with documented scoliosis had undergone an initial laminectomy.

4.5.5. Conclusions

The less severe the presenting neurological deficits, the more likely patients are to have a complete recovery. The rate of neurological recovery was similar for patients treated with chemotherapy compared to those managed with surgical decompression and laminectomy. Fewer orthopaedic sequelae were seen in the children managed with chemotherapy than were observed in children managed with laminectomy.

4.6. Memorial Sloan-Kettering cancer Center (by Kim Kramer, New York, USA)

We recently reviewed the cases of epidural neuroblastoma using neurological outcome and spinal deformities as endpoints [12]. From 1987 to 1998, 46 children with neuroblastoma invading one or more neural foramen by MR or CT with or without myelography were identified. Children were 7 weeks—18 years of age (median 3 years). Twenty-four patients (52%) were male. Two-thirds of patients had high-risk stage 4 disease by INSS criteria; the remainder 15 had loco-regional disease. Among 32 patients in whom epidural disease was discovered at initial presentation, the majority of tumours were of primary retroperitoneal origin, with a smaller percentage from the thoracic or pelvic region. Presenting signs or symptoms among the 46 patients included

pain (20 cases), weakness or gait disturbance (14), incontinence (3), sensory abnormalities (3), scoliosis (1) or were asymptomatic (13). Some patients had more than one symptom.

4.6.1. Therapy

A sole surgical approach was primarily reserved for patients with asymptomatic loco-regional disease; symptomatic patients with loco-regional disease were treated either by surgery or combination chemotherapy. Combination chemotherapy was favoured for patients with metastatic disease; those with stage 4 disease with associated neurological deficits were treated with both chemotherapy, chemotherapy and radiotherapy, and surgical decompression.

4.6.2. Neurological outcome

All patients with loco-regional neuroblastoma who were initially treated with surgery remained stable or improved neurologically. Five of six patients with stage 4 disease with associated neurological deficits treated surgically remained stable or improved; one paraplegic patient did not improve. Twelve stage 4 patients with neurological deficits were treated with chemotherapy, or chemotherapy and radiation therapy; nine remained stable or improved and three had neurological deterioration requiring emergent neurosurgery; all three subsequently improved.

4.6.3. Spinal deformities

Overall, 11/46 patients (24%) were noted to have spinal deformities (scoliosis and/or kyphosis) at some point (median, follow-up 5 years). Spinal deformities were rare in patients treated non-surgically (2/16). Age at diagnosis and median follow-up periods did not differ in patients who developed spinal deformities vs. those who did not.

4.6.4. Conclusions

Our experience shows that in patients with pre-existing neurological deficits, surgical intervention successfully stabilises or improves the neurological condition. Some stage 4 patients with neurological deficits may be treated with chemotherapy initially, but approximately 25% have progressive neurological deficits eventually requiring surgical intervention. Spinal deformity is a common late event in patients with epidural neuroblastoma, and is more frequently observed in patients treated with neurosurgical intervention (30%) compared to those treated with chemotherapy alone (12%). Low-risk neuroblastoma patients with epidural extension may be offered surgery alone, but the risk of scoliosis must be weighed against those of cytotoxic chemotherapy.

4.7. Japan (by Tomoko Iehara, Kyoto)

Children less than 12-month-old, presenting with neuroblastoma and spinal cord compression between June 1994 and May 2004, were enrolled into the Japanese prospective studies #9405 and #9805 [13]. Patients were treated with a conservative approach, avoiding neurosurgery, and prospectively followed-up. The aim of the study was to assess the efficacy of this treatment.

4.7.1. Patient demographics

Six hundred and fifty-seven cases of neuroblastoma were diagnosed in the study period. Thirty-three of them (5%) presented a dumbbell tumour. Twenty-two cases (67%) were detected by mass screening and 11 (33%) had symptoms. Nineteen patients were male, 14 female. Median age at diagnosis was 7 months. The primary tumour site was the thorax in 17 patients, abdomen in 14 and pelvis in 2. Three cases had stage 1, 10 had stage 2A, 2 had stage 2B, 12 had stage 3 and 6 had stage 4. None of the patients had *MYCN* oncogene amplification over 10 copies.

4.7.2. Symptoms and treatment

Nine patients had neurological symptoms at presentation, consisting of paraparesis in five cases (associated to sphincteric dysfunction in two), Bernard–Horner syndrome (CBHs) and dyspnea in three cases, CBHs and arm paresis in one case. No patients underwent neurosurgery. All symptomatic children were treated with chemotherapy. One patient received irradiation to the thoracic vertebrae. Patients with localized resectable disease underwent tumour excision (15 cases). Among them, stage 3 patients received post-operative chemotherapy (10 cases). Patients with localized unresectable tumours (12 cases) or stage 4 disease (six cases) underwent pre-operative chemotherapy. Fourteen of them also received post-surgery chemotherapy.

4.7.3. Outcome

No patients had neurological worsening. Complete neurological recovery was observed in two cases, and partial recovery in four. One patient, who had multiple malformations, died of disease. Five patients relapsed, but achieved a second remission. Twelve patients are alive with residual disease at 1–101 months of follow-up (median, 37). Twenty patients are alive with no evidence of disease (10–108 months; median, 65). Event-free survival of patients treated with surgery alone is 89%, of patients with localized disease who received post-operative chemotherapy is 80% and of patients with unresectable tumours or stage 4 disease, who underwent pre-operative chemotherapy is 80%.

4.7.4. Conclusions

The prognosis of infants with dumbbell-type neuroblastoma is good. Local control can be achieved without neurosurgery. A treatment plan without laminectomy can be considered adequate.

4.8. France (by Dominique Plantaz, Grenoble)

In 1990, a prospective study concerning children with localised neuroblastoma presenting with spinal cord compression was opened [14]. All these cases were treated with chemotherapy, in an attempt at reducing morbidity of laminectomy, while maintaining a good survival rate and offering the best chances of neurological recovery. Both symptomatic and asymptomatic patients were included into the study. Chemotherapy was administered according to NBL 90 and NBL 94 protocols. Decompressive neurosurgery was performed at diagnosis only in cases of initial severe and rapidly progressing neurological deficit. Patients enrolled into the NBL 90 protocol, but not those enrolled into NBL 94 protocol, underwent secondary laminotomy to remove intraspinal residue when appropriated.

4.8.1. Demographics

From 1990 to 1999, 618 cases of neuroblastoma were diagnosed. Seventy-eight of them (12.6%) had intraspinal extension. The median age at diagnosis was 8 months. Fifty-nine percent of the dumbbell patients were less than 12 months old, compared to 44% of non-dumbbell cases ($P = .04$). The proportion of thoracic tumours was significantly higher among dumbbell patients (45 vs. 24%; $P = .01$) and *MYCN* gene amplification was significantly less frequent (one of 57 cases in which *MYCN* status was studied, vs. 38 of 390; $P = .02$).

4.8.2. Results

Forty-four patients (56%) had some degree of neurological impairment, scored as partial in 24 patients (30%) and severe in 20 (26%). Thirty-eight of 44 patients (86%) were initially treated with chemotherapy. Of them, 24 (63%) had a complete and 8 (21%) had a partial neurological recovery, in 5 (13%) chemotherapy failed to improve the neurological status and one patient worsened. Intraspinal tumour regression was evaluated in 63 patients, both symptomatic and asymptomatic. Complete regression of the intraspinal tumour was observed in 50% of cases, while in 37% partial regression was described and in 11% no tumour shrinkage was noticed. Only in one case the intraspinal tumour progressed despite chemotherapy. Six symptomatic (14%) and two asymptomatic patients underwent primary neurosurgery. Only one of them was enrolled into the NBL 94 protocol. Fifty percent of symptomatic patients completely recovered, 33% partially improved and in one case surgery failed to improve patient's status. Thirteen patients, 10 of whom enrolled into NBL90 protocol, underwent secondary neurosurgery, for tumour residue (11 cases), worsening (1), or intraspinal relapse (1). Overall and event-free survival of dumbbell patients was comparable to non-dumbbell cases. The presence of an intraspinal residue did not correlate with risk of relapse. Forty-three of 44 symptomatic patients were evaluated for late sequelae. Seven cases (16%) presented mild, and 10 (23%) presented severe neurological sequelae (inability to walk and/or bowel/bladder dysfunction). Ten patients (23%) suffer from severe orthopaedic sequelae.

4.8.3. Conclusions

In France, initial laminectomy has become an uncommon procedure. Delayed laminotomy to remove an intraspinal residue after chemotherapy and extraspinal surgery has been performed, but can probably be avoided, without risk of jeopardizing the excellent survival of these patients. Although the diagnosis tends to be made earlier than in the past, antenatal or long lasting spinal cord compression still put about 25% of symptomatic patients at risk of life-long sequelae.