Table 5
Late sequelae

Type of sequelae	Cases		Neurological impairment		Treatment			
			Moderate	Severe	Laminotomy		Chemotherapy	
	#	%	#	#	#	%	#	%
Bladder dysfunction	12	52	5	7	10	67	2	25
Scoliosis	9	39	6	3	8	53	1	12
Orthesis	9	39	1	8	7	47	2	25
Reduced functional independence	8	40	2	6	7	47	1	12

Summary of the Italian experience.

5. Late effects (by Paola Angelini, Genova)

We have reviewed the Italian patients observed between 1979 and 2002. Patients were re-examined by MRI, orthopaedic, neurological, urologic and oncologic evaluations. The WeeFIM™ instrument was used to score the patients' functional independence. Both patients and parents were requested to separately answer a modified PedsQL™ questionnaire, adapted for children (6–12 years) and adolescents (13–18 years). Twenty-three patients, 13 male, 10 female, median age at diagnosis 8 months (range, 1–83), were evaluated. Twenty had a localized tumour, two had stage 4 and one had stage 4s disease. Motor impairment at diagnosis was mild in one case, moderate in 15, and severe in 7. Neurosurgery was the first therapeutic approach in 15 cases, chemotherapy in 8. Median follow-up was 7 years (range, 2–22). Table 5 summarises the results of our combined evaluation, with particular regard to bladder function, scoliosis, need for orthesis to walk, and age-adjusted functional independence. Six patients needed surgical correction of limb deformities, related to Achilles' tendon in two cases, hip in two, knee and feet in one case each. Eight patients had feet deformities. All patients answered the quality of life questionnaire. Parents tended to underweight some issues, mostly social and emotional. Anger and tiredness were the most commonly reported feelings. All patients described future, sometimes ambitious projects.

Overall, in 85% of patients relevant sequelae were documented, as a consequence of either epidural compression, or of its treatment. However, having more compromised patients been easier to contact and evaluate, an overestimation of the incidence and severity of sequelae might have occurred. The ascertainment of a correlation between presence and/or severity of sequelae and type of treatment, or severity of neurological impairment at diagnosis was difficult, as more severely affected patients received more aggressive treatment, often including laminectomy. The prospective collection of standardized and comprehensive data on larger series is warranted.

6. Conclusions (by Bruno De Bernardi, Genova, and Howard Katzenstein, Atlanta)

Symptomatic epidural compression in a child with a tumour is a medical emergency since severe sequelae may follow if the condition is not timely re-cognised and treated. Neuroblastoma is by far the tumour that most frequently present with such clinical pattern. A proportion of these cases despite a positive MRI have no symptoms arising the question if they require specific treatment. No doubt instead, that children with positive MRI and neurological symptoms do require prompt multidisciplinary attention. The contribution brought to this Workshop clearly shows that a number of disagreements still exist for a variety of aspects. In the conclusive part of this review, the controversies concerning these aspects will be summarised.

6.1. Definition

For sake of accuracy, the term spinal cord compression should be limited to the cases for whom the compression occurs just over the spinal

cord. However, in a proportion of the cases the compression involves the nerves and/or the cauda equina, without affecting the medulla. As a matter of fact, the clinical aspects, the neurological compromise and the eventual late effects may considerably differ in the two situations, being commonly less severe in the latter. The term epidural compression should therefore be preferable. In this review, the authors are have been free to use the term of their preference. In the future, an unified terminology will be desirable.

6.2. Incidence

Although it varies considerably in the different published series as well in the presentations of this Workshop, the incidence of cord compresssion seems progressively decreasing, possibly as an effect of early diagnosis. However, it has to be noted that some of these children receive chemotherapy on an emergency basis before a histologic diagnosis, making them ineligible for more recent trials. On the opposite, the inclusion of asymptomatic cases may definitely increase the incidence. Achieving an agreement on this issue appears of particular importance to compare different series of patients.

6.3. Clinical presentation

In analogy to previous series, children included in this review had more frequently localised disease, younger age and thoracic location. Since all these features are known to be associated with better outcome, not surprisingly children with epidural compression have a higher chance of survive than the general neuroblastoma population.

6.4. Definition of neurological deficit

Surprisingly, the description of neurological deficits in the various series lacks of common terms making it difficult to compare them for both clinical presentation, response to therapy and late results.

6.5. Imaging of epidural compression

MRI is definitely the instrument of choice to document the involvement of intravertebral foramina and spinal canal. However, it has still to be made clear if the presence of either involvement documented by MRI should not be sufficient to authorise the initiation of specific therapy in absence of neurological symptoms. The tendency emerged in the Workshop has been negative to this regard.

6.6. Time elapsed between symptoms and treatment

It is commonly believed that a long interval between first evidence of epidural compression and initiation of specific therapy is associated with worse clinical response and greater risk of relevant late sequelae. The data presented in this Workshop are uneven and controversial at this respect.

6.7. Therapeutic approach

Dexamethazone is often immediately administered but its benefit is controversial. Significant disagreement persist regarding the optimal specific therapeutic approach. While some consider that any case of documented epidural compression (even asymptomatic) should undergo a neurosurgical operation, others state the opposite. The presentations of this Workshop clearly document this noticeably different behaviour. Well organised prospective studies are needed to seriously face and solve this important issue.

6.8. Late effects

A large proportion of children with neuroblastoma presenting with epidural compression will develop significant sequelae, which may be especially severe in case of primary or secondary grade 3 neurological deficit (paraplegia) [15]. Once again, one is surprised of the scarcity of publications at this respect and lack of guidelines to follow to optimally treat these children in order to minimise the eventual sequelae.

6.9. Perspectives

The main aim of this Workshop was to collect on a large scale data on clinical and therapeutic aspects of epidural compression in neuroblastoma, this has allowed to document that a variety of aspects are not well defined and therefore require clarification. The participants have agreed on the necessity of planning an additional Workshop to fix common guidelines.

6.10. Rationale for an international neuroblastoma cord compression registry

Over the past two decades, uniform criteria for staging neuroblastoma have been developed (INSS), and discussions are ongoing to develop to uniform international neuroblastoma risk-group (INRG) criteria. The international neuroblastoma community has long recognized that a universal language is needed to compare results of clinical trials that are conducted in the various cooperative groups and countries throughout the world to insure that treatment strategies are optimised. As evidenced by this mini-review, the management of spinal cord compression in neuroblastoma remains controversial. Reviews of published series from largely describe retrospectively collected data, and to date, there are no uniform criteria for defining the type or severity of the neurological deficits that are associated with cord compression. In addition, criteria for evaluating neurological response to therapy are lacking. A better understanding of neuroblastoma and cord compression could be obtained through an international registry. Such a registry would allow for the prospective collection of data and would provide a uniform language to describe the neurological symptoms of these patients. As evidenced by the important contributions of the other international neuroblastoma collaborations, the creation of an International Neuroblastoma Cord Compression (INCC) Registry would facilitate the collection of a complete and uniform set of data which would hopefully lead to the development of an evidence-based approach to this problem.

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巻頭論文

標準的な小児がんの診断、治療構築目指す

国立成育医療センターでの放射線治療の取り組み

国立成育医療センター
放射線診療部

正木英一
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鹿島恭子
宮坂実木子
大楠郁子
岡本礼子

国立成育医療センターでは放射線診療部がすべての患者の画像診断を行っており、小児がん患者の診断に必要なモダリティの選択を臨床各科に推挙し、stagingに至る画像診断を行っている。

前施設である国立小児病院時代より行われている腫瘍カンファレンスを、Tumor Boardとして小児腫瘍科の主催のもとに外科、臨床検査部病理および放射線診療部（放射線治療科、放射線診断科）、時に主治医である総合診療部、脳神経外科、耳鼻咽喉科、整形外科、眼科、皮膚科が参加し、放射線診療部カンファレンス室にて、初診時の小児がん患者の診断・stagingを行い、治療法を決定している。

国立成育医療センター外観

EMBに基づいた放射線治療を他科医に提案できるシステム導入

国立成育医療センターで行っている小児がん放射線治療

1. 小児がん患者へのインフォームド・コンセント

国立成育医療センターではチーム医療を治療の主体としているので、その患者および腫瘍に関わる総合診療部、専門診療部の医師および看護師同席で患者家族に治療法の説明と予想されるその結果などを説明する。その後、小児であっても理解できる子どもに対しては、小児腫瘍医とともに年齢に応じた説明を両親同席のもと行っている。

さらに、両親に対して放射線治療の詳細に関すること、現在の標準的な治療法をPDQ日本語版など教科書的なもの、治療研究であればその資料を提示しながら主治医である小児腫瘍医とともに説明し、理解してもらっている。その場で、放射線晩期合併症についても起こり得ることを説明し、家族が主体的に治療に参加することが小児がんでは重要であることの理解を得ている。

また、治癒した後の患児が社会から受けるであろう事態（精神的あるいは身体的いじめ）から家族が守る必要性など、ソーシャルワーカーを交えての説明になることもある。

2. 患者の固定法

小児がん患児の放射線治療において、放射線治療ベッド上で体動なく照射野の再現性の良いと思われる年齢層は、小学校高学年以上である。その年齢層以下の小児では何らかの処置が必要とされている。通常は鎮静剤の投与によることが多く、1ヵ月近くもかかる分割照射では主治医である小児科医がその対応に追われて困難性を訴えることが多い。我々は国立小児病院時代から液晶テレビにて患児の好きなアニメーション・ビデオを見せることにより、2歳以上であれば無鎮静で放射線治療ができることを実証してきた。しかし、2歳以下においては麻酔科医が全身麻酔を行い、安全性の確保に努めている。

頭頸部腫瘍であればシェル・マスクを作成し、体幹部固定装置を用いることにより、さらに体動なく安全に放射線治療が行える環境を整えている（図1）。

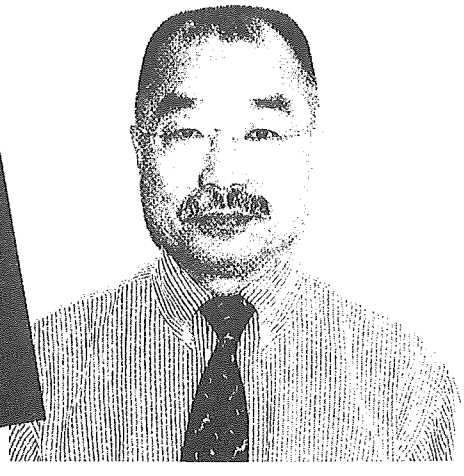
これに先立ち、患児が放射線治療室に慣れるよう、放射線治療は痛くないことを実感してもらう「模擬照射」期間を設けている。

3. CTシミュレーション

患児が放射線治療室に慣れたところで、固定装置を装着し、放射線治療室内に設置してあるCTシミュレーション装置にて腫瘍部を撮影する。

即ち、患者の初診時から放射線腫瘍医が診断および治療に積極的に関わり、治療のどの時期に放射線治療を採用するかということの適応条件を提示する。また、放射線治療の適応ではない疾患あるいはstageに関しても的確な助言を与える。これらのことは、従来より「化学療法が効かなくなったから放射線治療でも行う」といった、放射線腫瘍医からすると放射線治療という武器を有効に使わない考え方を全く払拭することにある。

このTumor Boardを形成することにより、放射線治療を有効な時期に集学的治療に取り入れ、腫瘍線量をなるべく少なくできる治療法を採用することが可能となる。化学療法と同時併用とするのか、化学療法後に放射線治療を行うのかといった判断をEBMに基づいて小児腫瘍医および小児外科医に提案するこのシステムは、小児がんだけではなくがん治療全般で行われるべきものである。



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●47年香川県生まれ。73年慶大医卒。同大医学部訓練医、助手を経て、82年専任講師（医学放射線科学）。同年国立小児病院放射線科医長、02年から国立成育医療センター放射線診療部長として現在に至る。慶大客員助教授、東邦大客員講師兼任。

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図1 小児の固定法

子どもの好きなアニメを液晶テレビで見せ、無鎮静で放射線治療を行う。

全脳照射時などでは液晶テレビを尾側に配置し、眼球を下方へ偏位させることにより水晶体保護が可能となる

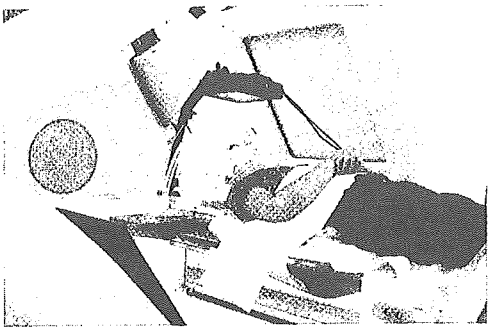


図1a 脳腫瘍におけるシェル・マスク固定法

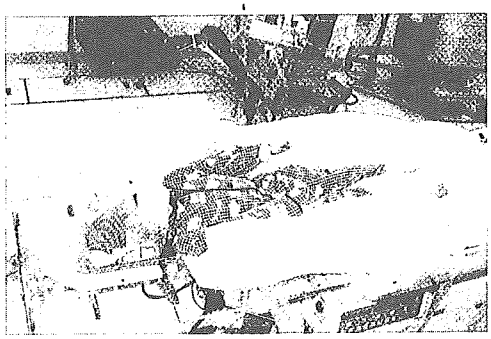


図1b 髄芽腫全脳全脊髄照射時におけるシェル・マスクおよび体幹部固定法（原発巣への局所照射療法中）

4. 3D治療計画

CTシミュレーション装置にて撮影したデータを3D治療計画装置に転送し、腫瘍巣をCTイメージに描画する。腫瘍の種類によって定められているGTVを描画するためには、どの時期の腫瘍巣を放射線治療のターゲットとするのかを理解しなければならない。初診時、化学療法後あるいは手術時の腫瘍巣をターゲットとする治療であるのかということである。

5. 各腫瘍における放射線治療法の選択：EBMを検索、あるいは治療研究への参加

小児がん各々において、欧米で行われてきた治療研究の成果を踏まえた本邦における治療研究が近年行われてきている。ウイルムス腫瘍はNational Wilms' Tumor Study（NWTS）、横紋筋肉腫はInternational Rhabdomyosarcoma Study（IRS）に準じた全国治療研究が行われており、各々に放射線治療ガイドラインが策定されている。

我々の施設を中心に全国の小児がんに積極的に取り組んでいる施設から放射線腫瘍医に参加を求めて、日本放射線腫瘍学研究グループ（Japanese Radiation Oncology Study Group：JROSG）の中の専門委員会、即ちJROSG小児放射線治療委員会として横紋筋肉腫放射線治療ガイドラインを策定し、神経芽腫放射線治療ガイドライン案を準備している。この全国治療研究に際し、小児がん放射線治療精度を高めるために放射線治療データセンターを国立成育医療センター放射線診療部内に設置した。

このセンターでは放射線治療後に放射線治療症例報告書CRFを収集し、それを解析することになっているが、セントラル・レビューセンター的な機能として放射線治療プランニング当初に相談を受け付け、リアルタイムにアドバイスが行えるシステムを構築している。また、治療研究が行われていない腫瘍群においては、これらの腫瘍に関しての原典となるPediatric Oncology Group（POG）などの文献および国立小児病院での経験に基づいたコンサルテーションを行い、小児科医および放射線腫瘍医に理解してもらうこととしている（masaki-h@ncchd.go.jp宛に連絡いただく）。

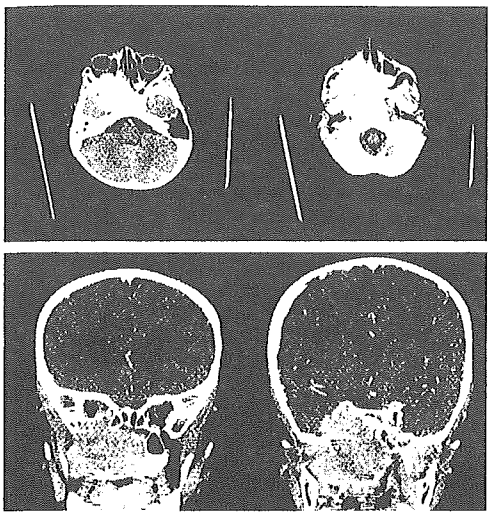
6. 小児がんに対する放射線治療の適応

放射線治療に感受性が高い小児がんが多く、最近の集学的治療ではウイルムス腫瘍、神経芽腫の術後照射線量は晩期合併症を心配する必要がない程度まで減量されてきている。これは化学療法のdose intensityを上げる試みが近年行われており、骨髄破壊的化学療法までも採用されてきたことによる。

しかしながら、横紋筋肉腫、脳腫瘍に代表される大線量を術後照射線量として要求されている小児がんにおいては、正常組織の障害を少なく、かつ腫瘍巣に線量を多く与えたいとしてhyperfractionationあるいは術中照射などが採用されている。hyperfractionationにおいては横紋筋肉腫、脳幹部腫瘍で治療研究が行われてきたが、通常分割照射法と比較して優位な効果を得ていない[1,2,3]。

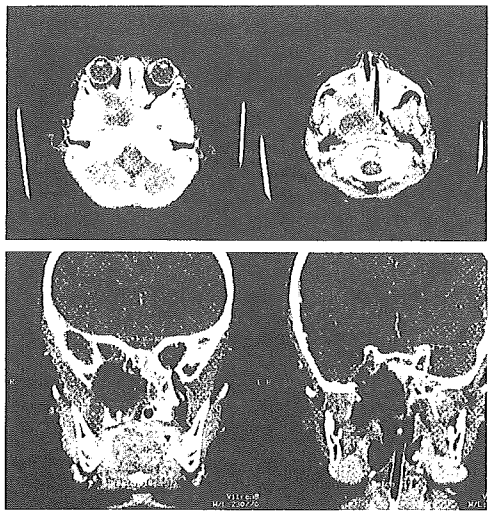
神経芽腫で用いる術中照射では、顕微鏡的

残存腫瘍に対して電子線エネルギー6MeV 10Gyを我々は採用している。この1回10Gyという線量は、抗腫瘍効果としては16Gy/8分割、正常組織における晩期障害の観点からは30Gy/15分割に相当する。そこで、慎重に的確な腫瘍巣の判定を行い、術中照射の利点、欠点をよく理解して適応を決める必要がある。この適応には、小児の鎮静の問題も介在する。分割照射では主治医である小児科医が毎日苦労して鎮静を行っているのが現状であり、薬剤による呼吸抑制などのリスクの中で放射線治療を行っている。このリスクを避けるために術中照射を採用することも、小児においては適応基準と考えられている。



coronal view

図2a 初診時造影CT axial view



coronal view

図2b 放射線治療6日後造影CT axial view

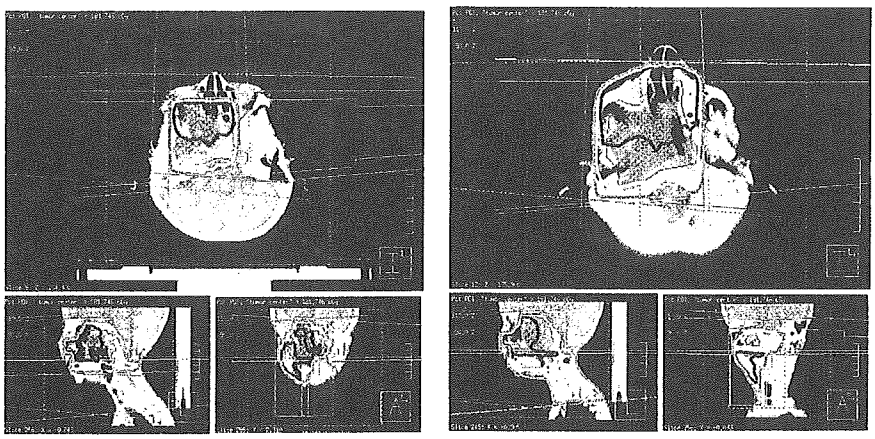


図2c 放射線治療線量分布図

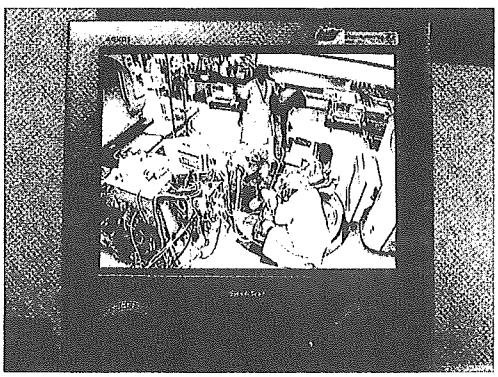
図2 横紋筋肉腫embryonal type（副鼻腔原発）2歳9ヵ月男男児　stage 3、group Ⅲ

鼻閉、右眼瞼下垂、右眼球突出にて来院。CT、MRIにて頭蓋底を破壊する上咽頭・副鼻腔腫瘍を認める。CT施行（図2a）後放射線治療開始し、化学療法IRS-IV　regimen46（VIE）を同時併用。照射開始6日目CTでは腫瘍に低吸収域が出現し、緊急照射による効果が現れた（図2b）。鼻腔原発巣には前左右3門，右頚部リンパ節転移部には前後2門とした（図2c）。化学療法12クール終了後、highMEC+THP-ADR前処置による自家末梢血幹細胞移植施行し、腫瘍は縮小し瘢痕組織を認めるのみとなった

7. 晩期合併症の認識

成長期にある小児に放射線治療を行うことに関しての認識を強くしなければならない。成人でいわれている正常組織耐容線量（表1）よりも通常は低い線量で合併症が発生することを考慮し、照射線による骨格および軟部組織の変形を最小限度許容できる程度に抑える照射方法を採用する。成人においては腫瘍巣に線量集中を行う定位放射線治療が盛んに行われているが、小児、特に頭頚部腫瘍において行うことは十分に注意する必要がある。

腫瘍巣に線量集中させたいと考えるのが通常であるが、顔面であれば照射後左右非対照の成長が起こり、著しい顔面変形が起こるこ



全麻下全身照射準備中のモニタリング：この状態を患者家族に見せている

図3 放射線治療中の放射線操作室内にて家族と一緒に患者のモニタリングを行う。患児を安心させるため、マイクを通して家族から声を掛けてもらうこともある

表1 放射線治療に対する正常組織耐容線量（成人）

臓器	TD5/5 容積 1/3	TD5/5 容積 2/3	TD5/5 容積 3/3	TD50/5 容積 1/3	TD50/5 容積 2/3	TD50/5 容積 3/3	評価項目
腎	5000	3000*	2300	—	4000*	2800	臨床的腎炎
膀胱	N/A	8000	6500	N/A	8500	8000	膀胱萎縮症状、容積減量
骨：							
大腿骨頭	—	—	5200	—	—	6500	壊死
顎関節	6500	6000	6000	7700	7200	7200	関節機能の著しい制限
肋軟骨	5000	—	—	6500	—	—	病的骨折
皮膚	10cm²	30cm²	100cm²	10cm²	30cm²	100cm²	毛細血管拡張症
	—	—	5000	—	—	6500	
	7000	6000	5500	—	—	7000	壊死、潰瘍
脳	6000	5000	4500	7500	6500	6000	壊死、梗塞
脳幹部	6000	5300	5000	—	—	6500	壊死、梗塞
視神経	部分容積なし		5000	—	—	6500	失明
視交叉	部分容積なし		5000	部分容積なし		6500	失明
脊髄神経	5cm	10cm	20cm	5cm	10cm	20cm	脊髄炎、壊死
	5000	5000	4700	7000	7000	—	
馬尾神経	容積効果なし		6000	容積効果なし		7500	臨床的に明らかな神経障害
上腕神経叢	6200	6100	6000	7700	7600	7500	臨床的に明らかな神経障害
眼：水晶体	部分容積なし		1000	—	—	1800	治療を要する白内障
眼：網膜	部分容積なし		4500	—	—	6500	失明
耳：中耳／外耳	3000	3000	3000*	4000	4000	4000*	急性滲出性耳炎
耳：中耳／外耳	5500	5500	5500*	6500	6500	6500*	慢性滲出性耳炎
耳下腺*	—	3200*	3200*	—	4600*	4600*	口腔内乾燥症（TD100/5：5000）
喉頭	7900*	7000*	7000*	9000*	8000*	8000*	軟骨壊死
喉頭	—	4500	4500*	—	—	8000*	喉頭浮腫
肺	4500	3000	1750	6500	4000	2450	間質性肺炎
心臓	6000	4500	4000	7000	5500	5000	心膜炎
食道	6000	5800	5500	7200	7000	6800	臨床的狭窄／穿孔
胃	6000	5500	5000	7000	6700	6500	潰瘍、穿孔
小腸	5000		4000*	6000		5500	閉塞、穿孔／瘻孔
大腸	5500		4500	6500		5500	閉塞、穿孔／潰瘍／瘻孔
直腸	100cm³容積効果なし		6000	100cm³容積効果なし		8000	重症直腸炎／壊死／瘻孔、狭窄
肝	5000	3500	3000	5500	4500	4000	肝不全

＊：50％以下の容積では著明な変化は認められない。
耐容線量：通常照射（1日1回180～200cGy、週5回法）での総線量
TD5/5：5年後に5％の確率で合併症が生じる線量
TD50/5：5年後に50％の確率で合併症が生じる線量

とは小児放射線治療を経験されている放射線腫瘍医であれば容易に理解できるであろう。このため、健側への放射線負荷を掛ける左右対向2門照射などが小児腫瘍においては、よく採用されるゆえんである（図2）。

8. 小児への放射線治療の特徴

我々の施設は小児がん症例が主体であり、年間放射線治療新患数が少ない施設であるがゆえに、患者および家族を接する時間が多く取れる余裕がある。毎回の放射線治療に放射線腫瘍医が立ち会い、セッティングの確認、ポータル・イメージの確認を行っている。放射線治療の際に、放射線操作室内に家族および看護師の同席を許可し、一緒にリニアック室内の患者の様子を見ることにしている（図3）。

成人がんの施設では、このような余裕がないのが実情であるので、これを推奨することはできないが、家族を患児とともに治療に参加させることの一助になると考えている。

標準的な治療構築の必要性

小児がん放射線治療における「標準治療」といわれるものは未だ存在しない。標準治療に近付くべく治療研究が行われ、その結果をもとに「標準的な治療」が教科書的に記載されている。少なくともその「標準的な治療」を患者および家族に提示し、選択できるようなシステムを構築することが「患者が望む放射線治療」になると考えている。

小児がん　第42巻第1号：64-68，2005

原　著

神経芽腫治療における外科治療の役割

（一施設症例の解析からの考察）

The role of surgical intervention in the treatment for neuroblastoma

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東　　真弓，竜田　恭介，田口　智章，水田　祥代
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要　旨

神経芽腫治療における外科治療の役割について一施設症例の解析から検討した．乳児神経芽腫においては，治療の主体は無理のない一期的外科的根治術であり，化学療法，放射線照射を避けることが可能であると思われる．年長児神経芽腫においては，化学療法後の外科治療と局所放射線照射により局所コントロールは可能であるが，遠隔転移のコントロールを含めて考えた場合，治療の主体は化学療法であり，外科治療はその前後の化学療法の妨げになるような手法は避けるべきと考えられた．

Key words：**神経芽腫，外科治療，生物学的特性**
neuroblastoma，surgical intervention，biology

はじめに

本邦における神経芽腫に対する全国規模の統一プロトコールは，1994年から確立された1歳未満の乳児神経芽腫プロトコール[1),2)]と1985年から確立された1歳以上の進行神経芽腫プロトコール[3),4)]に分かれており，乳児神経芽腫プロトコールは1998年に一度改訂され，進行神経芽腫プロトコールは，1991年と1998年に改訂されている．このような神経芽腫の2種類の統一プロトコールにおいて外科治療のカテゴリーは，診断時一期的根治術，生検，second look operartionによる根治術の3種類に大別される．

また，2004年3月にて本邦の国家事業としての乳児神経芽腫マススクリーニング検査[5),6)]は休止となり，乳児神経芽腫の診断，治療に関する概念は改変の時期であり，1才以上の進行神経芽腫治療に関しても，国際的に日本からの治療戦略，治療成績を発信できるような臨床研究確立のための準備が現在進行中である．以上のような本邦における神経芽腫治療の背景から，今回，乳児神経芽腫及び年長児進行神経芽腫の統一プロトコールに基づいて治療されてきた当教室における神経芽腫症例の解析を基に外科治療の役割と今後の全国規模の神経芽腫臨床研究における外科治療の展望について検討した．

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表1　1歳未満に発症した神経芽腫82例（1985－2003）

臨床像		症例数
病期	Stage 1, 2, 4S	70（85%）
	Stage 3, 4	12
MYCN増幅	非増幅	80（98%）
	増幅	2
転帰	生存	79（96%）
	死亡＊	3

＊1例のみ腫瘍死

対象と方法

当教室において1985年から2003年までに治療を開始した神経芽腫症例123例を解析対象とした．1才未満症例の82例中，73例がマススクリーニング症例であり，INSS分類[7]によるstage1,2,4S症例が79例(64%)であった．MYCN増幅に関しては，従来のサザンブロット法に加えてFISH法と定量的PCR法による解析を組み合わせて評価を行い[8]，増幅例（3コピー以上）は17例（14%）であり，現在，94例（76%）が生存している．

治療は原則として乳児症例は乳児統一神経芽腫プロトコールに従い，1才以上の進行症例は統一進行神経芽腫プロトコールに従って行った．基本的な外科治療指針としては，乳児神経芽腫症例に対しては，生検を含む臓器温存的腫瘍切除による初期手術を行い，Biologyが良好の残存腫瘍の腫瘍縮小後は，second look operationによる根治術は原則的には行なわなかった．また，1才以上進行症例に対しては，生検による初期手術を行い，腫瘍縮小後に，できるだけ臓器温存の系統的リンパ節郭清を行わないsecond look operationを施行した．その際，腎合併切除により腫瘍全摘が可能であれば，原則的に腎合併切除を行い，腎合併切除を行っても，主要血管浸潤等により腫瘍全摘が不可能の考えられた場合は，腎温存の腫瘍亜全摘を目標とした．その後，原則的には，腫瘍全摘であれば，体外放射線照射に加えて骨髄移植を伴う大量化学療法を行って治療を終了とし，また，腫瘍非全摘であれば，体外放射線照射に加えて地固め化学療法を続行した．

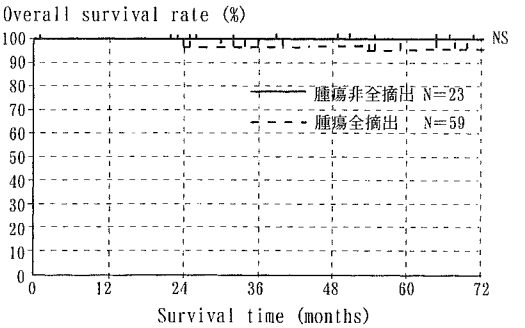

図1　1才未満症例における外科切除率と予後

各カテゴリーにおいてKaplan-Meier法を用いて生存率曲線を作成し，log-rank testによる生存率の有意差検定を行った．

結　果

乳児神経芽腫症例

乳児症例82例の臨床像を表1に示した．85%にあたる70例がstage1,2,4S症例であり，MYCN増幅例はわずか2例であり，98%が非増幅であった．死亡例は3例で，腫瘍死は1例のみであった．

原発巣の外科切除率と予後の関係では，98%が生存していることから，全摘出例と非全摘出例において有意差を認めなかった（図1）．また，乳児症例における化学療法施行と予後との関係では，全摘出例（59例）において化学療法の施行（61例），非施行（21例）に関して予後に差はなかった．非全摘出症例（23例）においては，全例に化学療法が施行されており，化学療法の有効性を評価できなかった（表2）．

表２　１歳未満症例における化学療法と予後

化学療法	外科切除率	生　存
施　行（N＝61）	全 摘 出（N＝38）	35（85％）
	非全摘出（N＝23）	23（100％）
非施行（N＝21）	全 摘 出（N＝21）	21（100％）
	非全摘出（N＝ 0）	

表３　１歳以上に発症した神経芽腫 41 例（1985－2003）

臨　床　像		症例数
病期	Stage 1，2	9
	Stage 3，4	32（78％）
MYCN 増幅	非増幅	26
	増幅	15（37％）
転帰	生存	15（37％）
	死亡	26

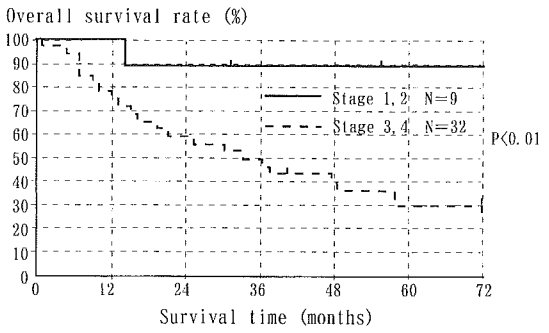

図２　１才以上症例における臨床病期と予後

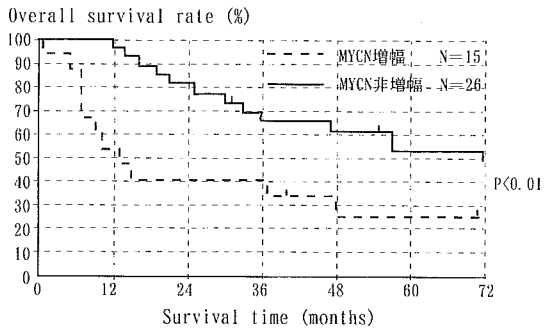

図３　１才以上症例における MYCN 増幅と予後

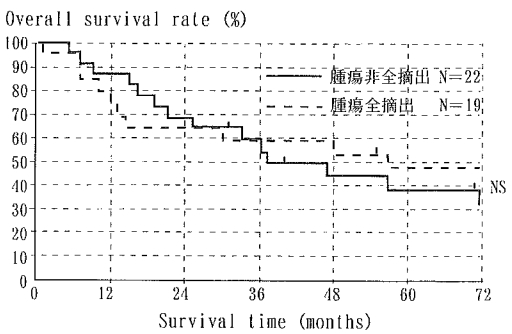

図４　１才以上症例における外科切除率と予後

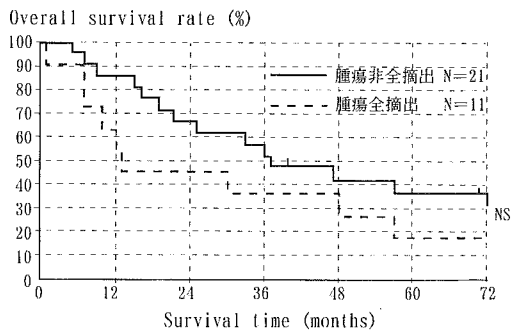

図５　１才以上進行症例における外科切除率と予後

表4　Stage 4で原発腫瘍全摘出症例の臨床経過（1994－）

症例	初診時転移部位	MYCN 増幅	術後局所照射	局所再発	転移再発	転帰
1	N，B，E，bm	非増幅	30Gy	(－)	B	腫瘍死
2	N，B，E，bm	増幅	30Gy	(－)	(－)	7年生存
3	N，bm	増幅	30Gy	(－)	(－)	4年生存
4	N，V，bm	増幅	30Gy	(－)	B，bm	腫瘍死
5	B	非増幅	30Gy	(－)	B，brain	腫瘍死
6	B，bm	増幅	30Gy	(－)	N，B，bm	腫瘍死

年長児（1才以上）症例

年長児（1才以上）症例41例の臨床像を表3に示した．78%にあたる32例がstage3,4の進行症例であり，MYCN増幅例が15例（37%）存在していた．現在の生存数は15例（37%）に過ぎなかった．

臨床病期と予後との関係では，stage1,2の早期症例（9例）は，有意差をもって，進行症例（32例）に対して予後良好であり（図2），MYCN増幅と予後との関係では，MYCN増幅症例（15例）は，非増幅症例（26例）に対して有意差をもって予後不良であった（図3）．

原発巣の外科切除率と予後との関係では，全摘出例（19例）と非全摘出例（22例）に有意差を認めず（図4），また，stage3,4の進行症例（32例）に限っても，原発巣の外科的切除率と予後との関係において，全摘出例（11例）と非全摘出例（21例）に有意差を認めなかった（図5）．

1994年以降のStage 4症例で原発腫瘍全摘出した6例の臨床経過を表4に示す．4例が再発しているが，再発形式は全て遠隔転移再発である．末梢血幹細胞移植に伴う大量化学療法は，このうち3例に施行したが，施行症例，非施行症例において再発形式及び転帰に差異を認めなかった．現行の統一進行神経芽腫プロトコールに基づいた導入化学療法と系統的リンパ節郭清までは行わない腫瘍摘出術に放射線照射を加えた治療により局所腫瘍のコントロールは良好であると言える．

考　察

乳児神経芽腫における外科治療の役割

マススクリーニング症例を中心とした乳児神経芽腫は大部分が局所腫瘍であり，悪性度も低く，外科切除率に関わらず予後良好であり，また，外科的に全摘出できた症例は化学療法の有無に関わらず予後良好であった．自験例において非全摘出例における化学療法非施行例が存在しないことから，非全摘出例における残存腫瘍への化学療法の必要性と有効性は評価できない．これらの結果からは，乳児神経芽腫における治療の主体は無理のない一期的外科的根治術であり，化学療法，放射線照射を避けることが可能であると思われる．

よって，今後の乳児神経芽腫における外科治療の展望としては，stage3，あるいは，stage2Aで初期手術が部分切除以下の症例に化学療法を省くことができるかと観点からの臨床研究が必要であると思われる．化学療法の有無に予後が関連なければ，初期手術はさらに臓器温存につとめ，腫瘍のbiologyの検索を重視する手術が推奨され，また，化学療法の有無が予後に関連するなら，初期手術においても，臓器温存を考慮したできる限りの腫瘍切除を重視するべきと考える．

また，本邦におけるマススクリーニングの休止により，今後の乳児神経芽腫症例は，マススクリーニング施行時期と比較して生物学的特性，及び臨床経過に変化がみられると予測される．治療全体におけるsurgical interventionによる腫瘍のbiologyの検索は乳児神経芽腫治療における最も重要な役割である．

年長児（1才以上）神経芽腫における外科治療の役割

今回の解析結果では，年長児神経芽腫においては臨床病期と腫瘍の悪性度が最も予後に相関して

おり，外科切除率と予後に有意な相関を認められなかった．また，米国の Children Cancer Group（CCG）の High risk NB における外科治療の効果に関する報告[9]においても，無病生存率において腫瘍全摘出例と非腫瘍全摘出例に有意差を認めていない．しかも，今回の症例においては，根治手術が，腫瘍非全摘出例において，より強力な化学療法が施行されたわけではなく，むしろ，腫瘍全摘出例に対して積極的に骨髄移植を伴う大量化学療法を選択する方針であったにも関わらず，全摘出例と非全摘出例に予後の差を認めなかったと言える．ただ，今回の解析における Stage 4 症例で原発腫瘍全摘出した 6 例の詳細な臨床経過の結果を考慮した場合，現行のプロトコールによる化学療法後の外科治療と局所放射線照射により局所コントロールは可能であったと考えられ，遠隔転移のコントロールを含めて考えた場合，治療の主体は化学療法であり，外科治療はその前後の化学療法の妨げになるような手法は避けるべきと考えられる．

今後，本邦において準備されている年長児進行神経芽腫に対する臨床試験における外科治療のコンセプトとしては，従来の局所根治を目指した手術の軽減化をはかり，外科治療による局所療法を可能な限り同一化することによりプロトコールの遂行性と効果の評価をより客観的にできることを目指すべきと考える．

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Biological diagnosis for neuroblastoma using the combination of highly sensitive analysis of prognostic factors

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BIN1;
Quantitative PCR

Abstract

Background/Purpose: To select the optimal treatment according to the degree of malignancy of neuroblastoma, it is essential to accurately and rapidly identify any genetic abnormalities associated with the prognosis. This study aims to assess the correlation between the combination of prognostic factors and the biologic findings of neuroblastoma using a highly sensitive analysis of prognostic factors.

Methods: In 44 neuroblastoma primary samples, we determined the gene dosages of *MYCN* and *Survivin* (as the target of 17q gain) and the expression levels of *MYCN*, *Survivin*, and *BIN1* using highly sensitive analysis (the quantitative polymerase chain reaction method); furthermore, we assessed the correlation between the combination of their prognostic factors and the biology of neuroblastoma.

Results: The gene dosage of *MYCN* or *Survivin* was significantly associated with all known prognostic factors. The expression level of *MYCN* or *Survivin* was not significantly associated with any prognostic factors, whereas the expression level of *BIN1* was significantly associated with 5 of 6 prognostic factors. Regarding the combination of *MYCN* amplification and 17q gain (the gene dosage of *Survivin*), and the low expression of *BIN1*, the rates of advanced stages (stage III or IV) were 100% for the cases with 3 factors, 63% for the cases with 2 factors, 42% for the cases with 1 factor, and 0% for the cases with null factor. Furthermore, the survival rates were 20% for the cases with 3 factors, 50% for the cases with 2 factors, 100% for the cases with 1 factor, and 100% for the cases with null factor.

Conclusion: The combination of gene dosages of *MYCN* and *Survivin* and the expression level of *BIN1* using the quantitative polymerase chain reaction method was significantly correlated with the clinical stage and the patients' outcome. This combination of biologic factors may enhance the accuracy to the conventional criteria, but this would have to be shown in a much larger study that is adequately powered to detect such an advantage.

Neuroblastoma is the most common solid tumor in children, and its development is still unclear [1]. The prognosis in neuroblastoma tends to vary greatly, and many studies have demonstrated both clinical and biologic

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factors to be correlated with the outcome [2]. To select the optimal treatment according to the degree of malignancy of neuroblastoma, it is essential to accurately and rapidly identify any genetic abnormality associated with the prognosis.

The amplification of the *MYCN* gene is the most unfavorable prognostic factor in neuroblastoma [3]. Approximately 20% to 30% of all patients presenting at advanced stages show an amplification of the *MYCN* gene, using the Southern blot method [4]. Regarding the *MYCN* gene, it is easy to consider that the amplification of *MYCN* gene results in an enhanced expression of *MYCN*, which activates the transcription of genes associated with the cell proliferation [5]. However, the clinical significance of *MYCN* expression in children with neuroblastoma remains controversial [6,7]. On the other hand, a gain of the chromosome 17q region has recently been implicated in close correlation with the aggressiveness of neuroblastoma, using either a comparative genomic hybridization study or the fluorescence in situ hybridization (FISH) method [8]. In particular, the gain of the q arm only has also been reported to play a role in an unfavorable outcome [9]. The region has been narrowed down to the 17q21-terminal, which is also considered to include the *Survivin* gene [10]. *Survivin* is a family member of an inhibitor of apoptosis proteins, and its expression is also cell cycle–regulated [11]. Recently, a high-level expression of *Survivin* in advanced stages of neuroblastoma has been shown, and it is thus considered to be one of the candidate genes for 17q gain [12]. We preliminary reported the quantitative polymerase chain reaction (PCR) method (TaqMan method) to be useful as a quick and accurate modality for evaluating for the status of *MYCN* amplification and the gene dosage of *Survivin* as the target of 17q gain in 25 neuroblastoma samples [13].

BIN1 (2q14) encodes multiple tissue-specific isoforms of an Myc-interacting adaptor protein that has features of a tumor suppressor, including the ability to inhibit Myc-mediated cell transformation and promote apoptosis [14]. We previously hypothesized that *BIN1* may function as a suppressor gene in neuroblastoma because *BIN1* is highly expressed in neural tissues and binds Myc within a region with 100% identity to *MYCN*, and reported data correlating reduced expression of *MYCN*-interacting *BIN1* isoforms with unfavorable features in primary neuroblastoma [15].

The quantitative TaqMan PCR System determines the initial copy number of the target gene by a kinetic analysis of the cycle-to-cycle change in the fluorescence signal as a result of the amplification of the template during PCR. Using this system, the detection of a loss of the 18q21 region in colon carcinoma tissue [16] and sex determination by defining the copy number of X chromosome have been reported. The advantage of quantitative PCR over Southern blotting, FISH, and comparative genomic hybridization is its speed (it takes about 4 hours from DNA extraction to the end of data analysis). Furthermore, this method is considered to have a high sensitivity [17].

In this study, we determined altogether the gene dosages of *MYCN* and *Survivin* (as the target of 17q gain) and the expression levels of *MYCN*, *Survivin*, and *BIN1* using highly sensitive analysis (the quantitative PCR method) in 44 neuroblastoma primary samples; furthermore, we assessed the correlation between the combination of their prognostic factors and the biology of neuroblastoma.

1. Materials and methods

1.1. Clinical data of patients and biologic data of neuroblastoma samples

Patients with neuroblastoma evaluated at the Department of Pediatric Surgery, Kyushu University, were diagnosed and staged according to the International Neuroblastoma Staging System [18]. Forty-four frozen tumor samples were obtained from untreated patients with neuroblastoma. The characteristics of the patients were shown to be as follows. The sex of the patients was 26 males and 18 females, and the age at diagnosis ranged from 19 days after birth to 11 years of age. Of the 44 cases, 15 patients were diagnosed at older than 1 year, whereas the remaining 29 were diagnosed at younger than 1 year. Twenty-four patients were identified by a neuroblastoma mass screening system. Of the 44 samples, 29 were tumors from patients who were stage I, II, or IVS, whereas 3 were stage III and 12 were stage IV. Thirty-six patients are still alive, of whom 3 cases are still under treatment, whereas 8 patients have died of the disease. The follow-up period after treatment ranged from 1 month to 12 years. In all 44 samples, the status of *MYCN* amplification was also previously determined by the Southern blotting method. In 36 of 44 cases with a single copy of *MYCN* identified by Southern blotting, DNA ploidy was previously examined using flow cytometry in 32 cases. Twenty-three cases were triploid, whereas 9 cases were diploid or tetraploid. Regarding the histologic findings, all 44 cases were classified based on the Shimada classification [19]. Thirty-two cases showed a favorable histology, whereas the remaining 12 cases showed an unfavorable histology. Regarding International Neuroblastoma Risk Group (INRG), all 44 cases were classified by age, stage (International Neuroblastoma Staging System), the status of *MYCN* amplification (Southern blot), DNA ploidy, and Shimada classification. Thirty cases showed a not high-risk group, whereas the remaining 14 cases showed a high-risk group.

1.2. DNA or RNA extraction and complementary DNA synthesis

DNA was extracted from the frozen tumor samples using proteinase K and phenol. Isogen LS (Nippon Gene, Osaka, Japan) was used to extract total RNA, and reverse transcription was performed with a First-strand complementary DNA synthesis kit (Amersham Pharmacia, Uppsala, Sweden) using random hexanucleotide primers.

Table 1 The sequences of the PCR primers and TaqMan probes for quantitative PCR

Target gene	Forward primer	Reverse primer	TaqMan probe
MYCN	5′-CCC AGC GTG GTA GTC AAT GA-3′	5′-TTA ATG ACA AAG CCA TAA TCC ACA G-3′	5′-AGA ATG CGC ACA TGA TGC TAC ACG TTT CT-3′
Survivin	5′-GGG CTG CCA CGT CCA C-3′	5′-GTC GTC ATC TGG CTC CCA-3′	5′-TTC ATC CAC TGC CCC ACT GAG AAC GA-3′
p53	5′-GCC CTT ACT TGT CAT GGC GA-3′	5′-ATC CCA CAA CCC CTG CG-3′	5′-TGT CCA GCT TTG TGC CAG GAG CC-3′

1.3. Quantitative PCR (TaqMan)

As previously described, the *p53* gene was used as an internal control gene to obtain the gene dosage (*MYCN/p53*, *Survivin/p53*). The p53 gene is a tumor suppressor gene in which mutations or deletions are found in a variety of malignant tumors. However, no aberration of the p53 gene in neuroblastoma has ever been found, and the gene status in neuroblastoma is known to be stable [20]. The corrected gene dosage of the *MYCN* gene and *Survivin* gene was obtained based on the assumption that the mean gene dosage of 20 normal individual lymphocytes was 1.00. The mean ± 2SD of *MYCN* gene dosage of 20 normal individual lymphocytes was 1.00 ± 0.58. In this study, we evaluated that the *MYCN* amplified cells apparently present in the samples with a corrected gene dosage (*MYCN/p53*) of more than 2.0. The mean ± 2SD of *Survivin* gene dosage of 20 normal individual lymphocytes was 1.00 ± 0.40. In this study, we evaluated that the *Survivin* amplified cells apparently present in the samples with a corrected gene dosage (*Survivin/p53*) of more than 1.50. The primers and TaqMan probes for the *MYCN* gene, *Survivin* gene, and the *p53* gene were designed using the application-based primer design software Primer Express (Applied Biosystems, Foster City, Calif). The sequences of the PCR primers and TaqMan probes were shown in Table 1. Quantitative PCR was performed in a final volume 25 μL, and each sample was analyzed in duplicate. Each reaction mixture contained 0.1 pmol/μL TaqMan probe, 0.2 pmol/μL each primer, 1× TaqMan PCR master mix, and 10 to 50 ng DNA. Thermal cycling was started with a 2 minutes incubation at 50°C, followed by a first denaturation step of 10 minutes at 95°C, and then 40 cycles of 2-step PCR consisting of 95°C for 5 seconds and 60°C for 1 minute. The quantification of the *MYCN* gene was achieved by means of the ABI Prism 7700 Sequence Detection System (Applied Biosystems). Genomic DNA from 1 neuroblastoma with 90 copies of *MYCN* by Southern blotting method was serially diluted to establish the calibration curve.

1.4. Quantitative reverse transcriptase PCR (TaqMan)

The primers and TaqMan probes were designed to be located on exons 2 to 3 for *MYCN* messenger RNA (mRNA), exons 2 to 3 for *Survivin* mRNA, and on exons 9 to 11 for *BIN1* mRNA, hereby avoiding the amplification contaminating genomic DNA. *GAPDH* was used as an internal control gene to analyze the *MYCN* gene expression (*MYCN/GAPDH*). The sequences of the PCR primers and TaqMan probe were shown in Table 2. Polymerase chain reaction primer and TaqMan probe for GAPDH were purchased from ABI as a kit of TaqMan GAPDH Control Regent and Predeveloped TaqMan Assay Regents Control Kit. The quantitative reverse transcriptase polymerase chain reaction (RT-PCR) system was performed in the same manner as that for the quantitative PCR.

1.5. Statistical analysis

Fisher's Exact test was used to test the association between *MYCN* amplification (*MYCN/p53*, ≥2.0) or 17q gain (*Survivin/p53*, ≥1.50) and other prognostic factors. The expression levels of *MYCN* (*MYCN/p53*), *Survivin* (*Survivin/GAPDH*), and *BIN1* (*BIN1/GAPDH*) in the subgroups were represented by percentile (50%). A comparison of the gene dosage and expression in relation to clinical and genetic parameters was made using Mann-Whitney *U* test. Kruskal-Wallis exact test was used to test the association between the clinical stage or the patients' outcome and the combination of 3 prognostic factors.

Table 2 The sequences of the PCR primers and TaqMan probes for quantitative RT-PCR

Target gene	Forward primer	Reverse primer	TaqMan probe
MYCN	5′-GAC CAC AAG GCC CTC AGT ACC-3′	5′-TGA CCA CGT CGA TTT CTT CCT-3′	5′-CCG GAG AGG ACA CCC TGA GCG A-3′
Survivin	5′-GAC GAC CCC ATA GAG GAA CAT AA-3′	5′-GGG TTA ATT CTT CAA ACT GCT TCT TG-3′	5′-CGT CCG GTT GCG CTT TCC TTT CT-3′
BIN1	5′-AAG GCC CAG CCC AGT GAC-3′	5′-GAG CCA TCT GGA GGC GAA G-3′	5′-CGC GCC TGC AAA AGG GAA CAA GA-3′

2. Results

2.1. The gene dosages of MYCN and Survivin by the quantitative PCR method

Of the 36 samples with a single copy of *MYCN* based on the Southern blotting method, 33 samples showed the corrected gene dosage (*MYCN/p53*) to be less than 2.0, whereas the remaining 3 samples with more than 2.0 had tumors from patients with an advanced stage of disease (stages III and IV). Of the 3 samples with a dosage of more than 2.0, 2 cases died of the disease. In 8 cases with more than 2 copies of *MYCN* based on the Southern blotting method, the corrected *MYCN* gene dosages by the quantitative PCR were all more than 10.0. In most of these cases, the analytic value based on the quantitative PCR was shown to be a higher than based on a Southern blotting analysis. The relationship between the *MYCN* gene dosage and the known prognostic factors (age, clinical stage, Shimada classification, INRG) is shown in Table 3. The group of cases with a gene dosage of more than 2.0 were strongly associated with an age of older than 1 year at diagnosis ($P < .001$), advanced stage ($P < .001$), a Shimada unfavorable histology ($P < .001$), and a high-risk group ($P < .001$), which are all unfavorable factors.

The corrected *Survivin* gene dosages ranged from 0.55 to 4.00. Ten cases showed that the *Survivin* gene dosages were more than 1.50-fold, and 6 of 10 cases were dead of disease. On the other hand, 32 of 34 cases with the *Survivin* gene dosages of less than 1.50-fold were free of disease. The relationship between the *Survivin* gene dosage and the known prognostic factors (age, clinical stage, Shimada classification, INRG) is shown in Table 3. The group of cases with a gene dosage of more than 2.0 was strongly associated with an age of older than 1 year at diagnosis ($P < .001$), advanced stage ($P < .001$), a Shimada unfavorable histology ($P < .001$), and a high-risk group ($P < .001$), which are all unfavorable factors.

Furthermore, we analyzed 20 samples that are clinically detected but not detected through mass screening. The relationship between the gene dosages *MYCN* or *Survivin* and the known prognostic factors was with the same trends compared with the results for all 44 samples. The gene dosage of *MYCN* was significantly associated with all 4 factors ($P < .01$), and the gene dosage of *Survivin* was significantly associated with 2 factors (age and Shimada, $P < .05$).

2.2. The expression level of MYCN, Survivin, and BIN1 by the quantitative RT-PCR method

The relationship between the *MYCN* gene, *Survivin* gene, or *BIN1* gene expression level and prognostic factors is shown in Table 4.

The level of *MYCN* expression in cases with *MYCN* amplification (*MYCN/p53*, ≥2.0) had a trend toward higher than that of cases with no *MYCN* amplification (*MYCN/p53*, <2.0); however, this finding was not statistically significant ($P = .15$). Furthermore, the expression level of *MYCN* was not significantly associated with any other prognostic factor (age, clinical stage, Shimada classification, the gene dosage of *MYCN* and *Survivin*, and INRG).

The level of *Survivin* expression was not significantly associated with the gene dosage of *Survivin*. In addition, the expression level of *Survivin* was not significantly associated with any other prognostic factor (age, clinical stage, Shimada classification, the gene dosage of *MYCN* and *Survivin*, and INRG).

The expression level of *BIN1* was significantly associated with 5 of 6 prognostic factors. Regarding 5 prognostic factors except the factor of age, the level of *BIN1* expression in neuroblastoma with the unfavorable factor was significantly lower than that in neuroblastoma with the favorable factor.

In addition, we analyzed 20 samples that are clinically detected but not detected through mass screening. The

Table 3 Gene dosage of *MYCN* and *Survivin* in relation to clinical and biologic prognostic factors

Category	n	*MYCN/p53*		*P*	*Survivin/p53*		*P*
		<2.0	≥2.0		<1.5	≥1.5	
Age (y)							
<1	29	27 (93.1)	2 (6.9)	<.01	28 (96.6)	1 (3.4)	<.01
≥1	15	6 (40.0)	9 (60.0)		6 (40.0)	9 (60.0)	
Stage							
Stage I, II, IVS	29	28 (96.6)	1 (3.4)	<.01	27 (93.1)	2 (6.9)	<.01
Stage III, IV	15	5 (33.3)	10 (66.7)		7 (46.7)	8 (53.3)	
Shimada							
Favorable	32	30 (93.8)	2 (6.2)	<.01	30 (93.8)	2 (6.2)	<.01
Unfavorable	12	3 (25.0)	9 (75.0)		4 (33.3)	8 (66.7)	
INRG							
Not high risk	30	29 (96.7)	1 (3.3)	<.01	28 (93.3)	2 (6.7)	<.01
High risk	14	4 (28.6)	10 (71.4)		6 (42.9)	8 (57.1)	

Values are presented as n (%). *P* value was determined by Fisher's Exact test.

Table 4 Expression of *MYCN*, *Survivin*, and *BIN1* in relation to clinical and biologic prognostic factors

Category	n	*MYCN*/GAPDH 50 percentile	*P*	*Survivin*/GAPDH 50 percentile	*P*	*BIN1*/GAPDH 50 percentile	*P*
Age (y)							
<1	29	0.22	.78	0.30	.79	1.17	.08
≥1	15	0.25		0.32		0.41	
Stage							
Stage I, II, IVS	29	0.15	.36	0.28	.42	1.36	<.01
Stage III, IV	15	0.52		0.5		0.41	
Shimada							
Favorable	32	0.19	.51	0.29	.43	1.21	<.01
Unfavorable	12	0.44		0.41		0.21	
Survivin/p53							
<1.5	34	0.31	.20	0.40	.94	1.15	<.05
≥1.5	10	0.08		0.27		0.38	
MYCN/p53							
<2.0	33	0.15	.15	0.34	.79	1.50	<.01
≥2.0	11	0.52		0.25		0.07	
INRG							
Not high risk	30	0.15	.33	0.29	.35	1.31	<.01
High risk	14	0.49		0.41		0.21	

P value was determined by Mann-Whitney *U* test.

relationship between the expression level of *MYCN*, *Survivin*, and *BIN1*, and the known prognostic factors was with the same trends compared with the results for all 44 samples. The expression level of *BIN1* was significantly associated with 4 factors (clinical stage, Shimada classification, the gene dosage of *MYCN*, and INRG; $P < .05$).

2.3. Evaluation of biology for neuroblastomas using the combination of 3 prognostic factors

In the highly sensitive analysis of prognostic factors in this study, the gene dosage of *MYCN*, the gene dosage of *Survivin*, and the level of *BIN1* expression were significant prognostic factors. The relationship between these 3 unfavorable prognostic factors (*MYCN* amplification—*MYCN/p53*, ≥2.0; 17q gain—*Survivin/p53*, ≥1.50; the low expression of *BIN1*—*BIN1/GAPDH*, <1.0) and clinical behavior (clinical stage and outcome) is shown in Fig. 1. Regarding the combination of *MYCN* amplification and 17q gain, and the low expression of *BIN1*, the rates of advanced stages (stages III and IV) were 100% for the cases with 3 factors, 63% for the cases with 2 factors, 42% for the cases with 1 factors, and 0% for the cases with null factor ($P < .001$, trend test using Kruskal-Wallis exact test). Furthermore, the survival rates were 20% for the

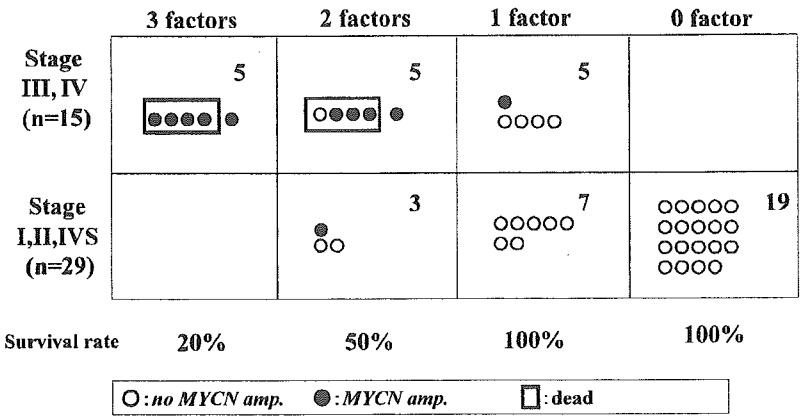

Fig. 1 The correlation of 3 unfavorable factors (*MYCN* amplification—*MYCN/p53*, ≥2.0; 17q gain—*Survivin/p53*, ≥1.50; the low expression of *BIN1*—*BIN1/GAPDH*, <1.0) and the clinical behavior in neuroblastomas. The rates of advanced stages (stages III and IV) were 100% for the cases with 3 factors, 63% (5/8) for the cases with 2 factors, 42% (5/12) for the cases with 1 factors, and 0% (0/19) for the cases with null factor ($P < .001$, trend test using Kruskal-Wallis exact test). The survival rates were 20% for the cases with 3 factors, 50% for the cases with 2 factors, 100% for the cases with 1 factor, and 100% for the cases with null factor ($P < .001$, trend test using Kruskal-Wallis exact test).

cases with 3 factors, 50% for the cases with 2 factors, 100% for the cases with 1 factor, and 100% for the cases with null factor ($P < .001$, trend test using Kruskal-Wallis exact test).

3. Discussion

Neuroblastomas have a variety of genetic variables that might predict the clinical behavior [2]. To select the optimal treatment according to the degree of malignancy of neuroblastoma, it is essential to accurately and rapidly identify any genetic heterogeneity associated with the prognosis. Generally, the gene dosage was analyzed by Southern blot method or FISH, whereas the expression level of gene was assessed by Northern blot method or semiquantitative PCR method. We previously reported that the quantitative PCR method may be considered to be the most effective methods for quickly and accurately evaluating any aberration in the gene dosages associated with the patients' outcomes [13,21]. In this study, we determined altogether the gene dosages of *MYCN* and *Survivin* (as the target of 17q gain) and the expression levels of *MYCN*, *Survivin*, and *BIN1* using highly sensitive analysis (the quantitative PCR method) in 44 neuroblastoma primary samples.

Regarding the *MYCN* gene, the amplification of the *MYCN* gene is strongly associated with rapid tumor progression [3,4]; however, the clinical significance of *MYCN* expression in children with neuroblastoma remains controversial [6,7]. In the present study, the gene dosage of *MYCN* was significantly associated with all prognostic factors, whereas the expression level was not significantly associated with any prognostic factor. Furthermore, the significant association between the gene dosage and the expression level was not observed. These findings are suggesting that the only gene dosage of *MYCN* does not always contribute to the level of *MYCN* expression in neuroblastoma, and the expression level of *MYCN* does not seem to be an independently significant prognostic factor in this highly sensitive analysis.

Regarding the *Survivin* gene, we assumed the *Survivin* gene could be one of the candidate genes for the 17q gain in neuroblastoma. In the present study, the gene dosage of *Survivin* was significantly associated with all prognostic factors, whereas the expression level was not significantly associated with any prognostic factor. In addition, there was no correlation between the *Survivin* gene dosage and the expression level. These results are demonstrating that analysis of the gene dosage of *Survivin* is useful for evaluating the 17q gain; however, the *Survivin* was not the candidate gene for 17q gain in neuroblastoma.

It is unclear why gene dosage is more correlative with risk than expression level. The chromosomal gain or loss may be correlated with the genomic instability, which is associated with poor prognosis in adult cancers [22]. The genomic instability may generate *MYCN* amplification or 17q gain as one of chromosomal alteration in neuroblastoma.

Taken together, the gene dosage of *MYCN* (*MYCN* amplification), the gene dosage of *Survivin* (17q gain), and the level of *BIN1* expression were significant prognostic factors in the highly sensitive analysis using the quantitative PCR method. Furthermore, the combination of gene dosages of *MYCN* and *Survivin* and the expression level of *BIN1* using the quantitative PCR method was substantially correlated with the clinical stage and the patients' outcome. The current protocol for neuroblastoma in the world is mainly based on the age, clinical stage, and *MYCN* amplification. In the Study Group of Japan for Advanced Neuroblastoma, 2 chemotherapeutic regimens for advanced neuroblastoma have been designed based on the *MYCN* amplification status since 1991 [23]. However, the status of *MYCN* amplification does not necessarily predict the patients' outcome. This combination of biologic factors may enhance the accuracy to the conventional criteria (*MYCN*, Shimada classification), but this would have to be shown in a much larger study that is adequately powered to detect such an advantage.

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