

# Localized unresectable neuroblastoma: results of treatment based on clinical prognostic factors

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**Background:** We previously reported that stage 3 neuroblastoma comprises (i) a low-risk group including all infants (age 0–11 months) as well as older children with non-abdominal primaries, and (ii) a high-risk group made up of children >1 year of age with abdominal primaries. Aggressive chemotherapy was effective only in the latter group.

**Patients and treatment:** On this basis, in 1990 we designed a new protocol by which all low-risk patients received standard-dose chemotherapy, while the high-risk ones received very aggressive chemotherapy.

**Results:** Between November 1990 and December 1997 a total of 95 eligible and evaluable children were enrolled: 47 were low-risk (35 infants and 12 >1 year of age at diagnosis and having non-abdominal primaries), and 48 were high-risk (being >1 year of age and having abdominal primaries). Of the 47 low-risk patients, five relapsed and four subsequently died. The 5-year overall survival (OS) was 91%. Of the 48 patients in the high-risk group, 22 relapsed or progressed, 18 of whom died from their disease and two from toxicity, and one was lost to follow-up. The 5-year OS was 60%. Univariate analysis showed that age, site of primary, risk-group, urine vanillylmandelic excretion, plasma levels of lactate dehydrogenase, ferritin and neurone-specific enolase, and *MYCN* status correlated with outcome. However, multivariate analysis showed that only *MYCN* status retained prognostic value.

**Conclusions:** In low-risk stage 3 neuroblastoma, standard-dose chemotherapy is associated with an excellent chance of being cured. Aggressive chemotherapy is effective for high-risk patients, but results are still unsatisfactory. *MYCN* gene amplification is a prognostic indicator for most, but not all, treatment failures.

**Key words:** prognostic factors, treatment, unresectable neuroblastoma

## Introduction

Neuroblastoma is the most common malignant tumor of early childhood. The median age at onset is ~2 years, and one-third of the patients are <1 year old when first seen [1]. The clinical course of neuroblastoma is influenced in an independent manner by two variables, namely age and disease extent at the

time of diagnosis. Infants (aged 0–11 months) have a much better outcome than older children, regardless of disease extent, and children with low disease stage (resected or almost resected primary tumors) are commonly cured by surgery alone, regardless of age [2, 3]. Understandably, patients who possess both unfavorable characteristics (approximately half of the cases) do particularly poorly, and their chance of being cured is <30% [4].

In the range from the almost benign to the almost incurable tumors there is a small population of patients who present unresectable tumor masses but who have no metastatic spread. The outcome for these patients is less predictable, as shown by the rather different results of the few recent publications

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reporting long-term survival as ranging from as low as 60% [5] to as high as 88% [6].

On behalf of the Italian Cooperative Group for Neuroblastoma (ICGNB), we previously reported that this population includes two subsets of patients with a distinctively different survival expectancy [7]: (i) a low-risk group that includes all infants and older children with non-abdominal primary tumors; and (ii) a high-risk group comprising children 1 year of age or older with primaries located in the abdomen. Rather interestingly, the use of an intensified chemotherapeutic regimen was effective in improving survival results in the high-risk patients but not in the low-risk ones. On the basis of these data, the treatment protocol designed in 1990 by the ICGNB for unresectable neuroblastoma consisted of standard-dose chemotherapy for patients with low-risk features, and very aggressive chemotherapy for patients with high-risk features.

The main aims of this study were (i) to both confirm that standard-dose chemotherapy is suitable for low-risk patients, and to possibly identify clinical and biological characteristics associated with eventual failure, and (ii) to evaluate whether the modifications made to the previous high-dose chemotherapy regimen actually led to further improvement of results.

## Patients and methods

### Patient population

This analysis refers to all children (age 0–15 years) with localized but unresectable neuroblastoma diagnosed between November 1990 and December 1997 in 22 Italian institutions participating in the ICGNB (see Acknowledgements). The criteria to define disease extension were those of both the ICGNB [7] and of the International Neuroblastoma Staging System (INSS) [8]. The tumors were defined as unresectable by a team of pediatric surgeons, diagnostic radiologists and pediatric oncologists after evaluation of clinical and imaging data. The definition encompassed: (i) tumors that crossed and infiltrated the midline, usually encasing large vessels; and (ii) tumors that were difficult to resect without a high risk of rupture or major surgical complications due to their large size and particular structure or location (including some thoracic tumors that did not clearly cross the midline but compressed and displaced the upper respiratory tract). Patients whose tumors had been evaluated as operable but who had undergone surgery leading to a macroscopic residue exceeding 2 ml were also included in the analysis, as were those with resected primary tumors but with histologically positive controlateral lymph nodes. Exclusion criteria included any antitumor therapy preceding enrollment into the study, and proof of severe organ impairment. Informed parental consent was obtained before beginning therapy.

Diagnosis was usually made on histological grounds. A few patients presenting features that strongly supported the hypothesis of neuroblastoma, but who were in poor general conditions making surgery risky, were included as well. Diagnostic work-up included evaluation of the primary tumor by computed tomography and/or magnetic resonance imaging, scintigraphic study with <sup>123</sup>I-metaiodobenzylguanidine, one to four bone marrow aspirates and at least one bone marrow biopsy (the latter was limited to children 1 year of age or older), and the assay of vanillylmandelic (VMA) and homovanillic (HVA) acids in the urine, and of

ferritin, neurone-specific enolase (NSE) and lactate dehydrogenase (LDH) in the serum.

Owing to the lack of revision of the histological material on a national level and the difficulties in classifying neuroblastoma on small and often scarcely representative biopsy specimens [9], the histological classification of tumor specimens was not taken into account when analyzing prognostic factors. Biological studies were performed on almost all tumor specimens sent to the national reference laboratory located in Genova [10].

### Patient stratification

Patients with unresectable neuroblastoma were divided into two groups according to the clinical factors we had previously found to correlate with outcome. The low-risk group included all infants (0–11 months) and older patients with primary tumors located in the thorax, neck or pelvis. The high-risk group was made up of patients 1 year of age or older, with abdominal primary tumors.

### Neo-adjuvant chemotherapy (Table 1)

Low-risk patients were treated with 3-monthly chemotherapy cycles made up of vincristine 1.5 mg/m<sup>2</sup> on day 1, cyclophosphamide 150 mg/m<sup>2</sup>/day orally on days 1–7 and doxorubicin 35 mg/m<sup>2</sup> on day 7. Patients achieving at least minor tumor response were entitled to receive three additional cycles, while patients with stable or progressive disease were switched to the high-risk treatment.

High-risk patients were treated with two consecutive protocols depending on when diagnosis was made. Patients diagnosed between November 1990 and December 1992 were treated using the ICGNB-89 protocol, for which induction chemotherapy consisted of two cycles of peptichemo 500 mg/m<sup>2</sup> followed by two cycles of cyclophosphamide 600 mg/m<sup>2</sup>, vincristine 1.5 mg/m<sup>2</sup> and cisplatin 200 mg/m<sup>2</sup> [11]. Patients diagnosed between January 1993 and December 1997 were treated using the ICGNB-92 protocol and thus received four cycles of deferoxamine 4 g/m<sup>2</sup> for the first cycle and 7.5 g/m<sup>2</sup> for the following three cycles, with cyclophosphamide 600 mg/m<sup>2</sup>, etoposide 300 mg/m<sup>2</sup>, thiotepa 30 mg/m<sup>2</sup> and carboplatin 1000 mg/m<sup>2</sup> [12].

All patients with a residual or still inoperable tumor after chemotherapy were treated individually with further chemotherapy or radiotherapy, or <sup>131</sup>I-metaiodobenzylguanidine (MIBG) therapy according to the characteristics of the residual tumors and to the decision of the referring physicians. Patients achieving complete response after chemotherapy received no further therapy.

### Surgery of primary tumor

Complete resection was defined as a radical resection or a resection leaving a residual tumor of <2 ml volume. Partial resection was a resection >50%, but less than complete. Biopsy was defined as a resection ranging from a fragment suitable for histological diagnosis to 50% of the primary mass.

### Definition of tumor response

Complete remission was defined as the disappearance of the primary tumor and return to normal values of VMA and HVA urinary excretion; partial response as a >50% decrease in the primary tumor; minor response as a 25–50% decrease in the primary tumor; no response as a <25% decrease or <25% increase of the primary tumor; and progressive disease as a >25% increase in the primary tumor or the appearance of a new lesion(s). Duration of response was not included in the definition.

**Table 1.** The ICGNB protocols for unresectable neuroblastoma 1990–1997

Protocol	Induction	Consolidation
Low-risk patients		
AIEOP NB 89 and 92	VCR 1.5 mg/m <sup>2</sup> day 1	Delayed surgery If CR stop therapy if <CR chemotherapy or radiotherapy or MIBG therapy
	DOX 35 mg/m <sup>2</sup> day 7	
	CPM 1050 mg/m <sup>2</sup> days 1–7 × 3–6 cycles	
	Surgery day 80 or ~170	
High-risk patients		
AIEOP NB 89	PTC 500 mg/m <sup>2</sup> days 1–5 × 2 cycles	Delayed surgery If CR stop therapy if < CR Cpt 800 mg/m <sup>2</sup> day 1–2 VP16 300 mg/m <sup>2</sup> day 1–2 2–4 cycles or radiotherapy or MIBG therapy
	VCR 1.5 mg/m <sup>2</sup> day 1	
	CPM 600 mg/m <sup>2</sup> day 1	
	CDDP 200 mg/m <sup>2</sup> days 1–5 × 2 cycles	
	Surgery day ~130	
AIEOP NB 92	Deferoxamine 7.5 g/m <sup>2</sup> days 1–5	VP16 300 mg/m <sup>2</sup> day 1–2 2–4 cycles or radiotherapy or MIBG therapy
	CPM-600 mg/m <sup>2</sup> days 5–6	
	TT 30 mg mg/m <sup>2</sup> days 5–7	
	VP16 450 mg/m <sup>2</sup> days 5–7	
	Cpt 1 g/m <sup>2</sup> days 6–7 × 4 cycles	
	Surgery day ~150	

CPM, cyclophosphamide; VCR, vincristine; DOX, doxorubicin; PTC, peptichemio; CDDP, cisplatin; VM26, teniposide; VP16, vesiposide; TT, thiotepa; Cpt, carboplatin; MIBG, <sup>131</sup>I-metaiodobenzylguanidine therapy; CR, complete remission.

### Evaluation of response to therapy

Tumor response was evaluated; (i) after induction chemotherapy, (ii) within 1 month after delayed surgery, (iii) 1–2 months after the end of therapy, and (iv) at least every 6 months for the following 5 years.

### Statistical analysis

The proportion of patients belonging to the two risk groups in different strata of prognostic factors was compared using the  $\chi^2$  test or Fisher's exact test. The following variables were investigated for their prognostic impact on overall survival (OS): gender, age at diagnosis (<1 year versus 1–15 years), site of the primary tumor (abdomen versus other sites), *MYCN* oncogene copy number (<3 versus  $\geq 3$  copies), VMA and HVA urinary excretion (<2.5 versus  $\geq 2.5$  SD), and serum values of LDH (<1000 versus  $\geq 1000$  IU/l), NSE (<100 versus  $\geq 100$  ng/ml) and ferritin (<150 versus  $\geq 150$  ng/ml).

OS was defined as the time between diagnosis and death, regardless of the cause. Event-free survival was defined as the time between diagnosis and relapse or progression. Follow-up times were truncated on 31 March 1999, 15 months after the last patient was enrolled. Estimates of OS and event-free survival were calculated according to the Kaplan–Meier product limit method. Comparisons of estimated survival curves were performed by means of the Mantel–Haenszel  $\chi^2$  test. The uni- and multivariate estimates of the hazards ratio among different strata were

calculated by Cox's proportional hazards model, and appropriate 95% confidence intervals were also reported. All tests are two sided.

### Results

Between November 1990 and December 1997, a total of 96 eligible patients with localized but unresectable neuroblastoma (all stage 3 according to both ICGNB and INSS staging criteria) were registered by 22 Italian institutions. They represent 17% of the 580 previously untreated neuroblastoma patients aged 0–15 years diagnosed over the same period (stage 1, 100 cases; stage 2, 86; stage 4, 242; stage 4s, 56). One patient was excluded from the analysis due to lack of information after diagnosis, leaving 95 evaluable patients, of whom 47 had low-risk characteristics and 48 had high-risk ones. Nine patients in the latter group had been treated using the ICGNB-89 protocol, and 39 using ICGNB-92.

### Patients' characteristics

The main clinical characteristics of the 95 evaluable patients are listed in Table 2. There was a slight prevalence of females (57 versus 43%). Median age at diagnosis was 19 months

**Table 2.** Localized but unresectable neuroblastoma; distribution of characteristics at diagnosis according to risk group

	Total	%	Low risk	%	High risk	%
Cases	95		47	49	48	51
Gender						
Male	41	43	14	30	27	56
Female	54	57	33	70	21	44
Age range [median, (months)]	0–150 (19)		0–146 (8)		12–150 (31)	
Site of primary						
Abdomen	72	76	24	51	48	100
Thorax	14	15	14	30		
Pelvis	5	5	5	11		
Neck	4	4	4	8		
VMA (92 tested)						
Normal (<2.5 SD)	24	26	10	22	14	30
Abnormal ( $\geq$ 2.5 SD)	68	74	35	78	33	70
HVA (81 tested)						
Normal (<2.5 SD)	20	25	11	29	9	21
Abnormal ( $\geq$ 2.5 SD)	61	75	27	71	34	79
LDH (92 tested)						
<1000 UI/l	59	64	36	80	23	49
>1000 UI/l	33	36	9	20	24	51
Ferritin (80 tested)						
<150 ng/ml	56	70	28	72	28	68
>150 ng/ml	24	30	11	28	13	32
NSE (65 tested)						
<100 ng/ml	42	65	26	84	16	47
>100 ng/ml	23	35	5	16	18	53
MYCN oncogene (80 tested)						
<3 copies	68	85	38	95	30	75
$\geq$ 3 copies	12	15	2	5	10	25

(range 1 day to 13 years). The abdomen was by far the most common site of primary tumors (72 cases), followed by thorax (14), pelvis (five) and neck (four). Diagnosis of neuroblastoma was made on histological grounds in 88 cases, and on imaging studies, elevated urinary catecholamines and an appropriate clinical pattern in seven patients, five of whom subsequently underwent surgery confirming the clinical impression of neuroblastoma. The majority of patients had elevated urinary catecholamines but normal plasma levels of LDH, NSE and ferritin. Of the 80 patients who were evaluated for *MYCN* oncogene copy number, 12 (15%) had three or more copies of the gene.

Among the 47 patients with low-risk characteristics, 35 (74%) were <1 year of age and had primary tumors were located in the abdomen (24 cases), thorax (five), pelvis (two) or neck (four). The primary tumors of the 12 remaining patients  $\geq$ 1 year of age were located in the thorax (nine cases) or pelvis (three).

Forty-eight patients >1 year of age with abdominal primaries made up the high-risk group. Comparing low-risk patients with high-risk patients, the latter showed a greater male to female ratio and a higher percentage of abnormal values for LDH and NSE concentrations, and *MYCN* oncogene copy numbers (Table 2).

### Clinical course

#### Low-risk group

Among 47 patients, 40 underwent biopsy and four underwent partial tumor resection. The remaining three patients were not operated on at this time. With the exception of an infant with a cervical tumor who died early of locally progressive disease, all patients were evaluated for tumor response following the six cycles of induction therapy. Complete response was observed in 10 patients, partial in 28 and minor in eight.

Following induction chemotherapy 25 of 46 evaluable patients underwent surgery, which resulted in complete resection in 15, partial resection in eight, biopsy in one and lack of visible tumor in another. The remaining 21 patients were not operated on because they were in complete remission (10 cases), or because their tumors still appeared inoperable (11 cases).

In conclusion, after chemotherapy and surgery (if performed), 26 patients were disease free and 20 had residual tumors (17 having achieved partial remission and three minor remission). None of the 26 patients in complete remission received further therapy. Twenty-five of them persist in remis-

sion while one, who had an amplified *MYCN* gene, relapsed locally and died. Of the 20 patients with residual tumors, five received therapeutic doses of MIBG (no relapse) two underwent additional chemotherapy (no relapse) and 13 had no further treatment (three local relapses). The characteristics of patients who relapsed or progressed are summarized in Table 3.

Overall, 41 of 47 patients are presently progression free at 11–90 months from diagnosis (median 40 months). Five patients relapsed or progressed at the primary tumor site, of whom four died, and one is alive in third remission. One patient was lost to follow-up.

**Table 3.** Characteristics of patients who relapsed or progressed

Case	Sex/age (months)	Primary site	VMA value	HVA value	LDH (IU/l)	Ferritin (mg/ml)	<i>MYCN</i> copies	Status after induction + surgery	Relapse/time (months)	Outcome
Low risk										
1	F/5	Neck	N	nd	nd	5	nd	PD	Local/1	DPD
2	F/2	Thorax	nd	nd	nd	nd	3	PR	Local/3	DPD
3	F/4	Neck	N	N	758	154	1	PR	Local/21	ADF
4	M/7	RPG	N	N	8200	314	100	CR	Local/4	DPD
5	M/49	Thorax	E	E	448	79	1	MR	Local/9	DPD
High risk										
1	M/53	Adrenal	E	E	3540	83	1	MR	Local/18	DPD
2	F/15	RPG	N	N	5910	191	14	PR	Disseminated/25	DPD
3	M/150	Adrenal	N	N	763	393	nd	MR	Both/7	DPD
4	M/40	Adrenal	E	E	1012	239	1	CR	Both/23	ADF
5	M/43	Adrenal	E	E	9140	969	24	CR	Local/24	DPD
6	M/73	Adrenal	N	N	367	41	1	PR	Disseminated/15	AWD
7	F/51	Adrenal	E	E	960	243	14	MR	Disseminated/15	DPD
8	F/138	Adrenal	E	E	435	97	1	CR	Both/34	AWD
9	F/30	Adrenal	N	N	1038	62	1	CR	Local/15	ADF
10	F/91	RPG	N	N	2130	132	8	PR	Both/9	DPD
11	M/14	RPG	N	N	990	750	1	MR	Local/7	DPD
12	M/25	Abdomen	E	E	3680	18	1	PD	Local/1	DPD
13	M/28	Adrenal	E	E	1653	400	20	CR	Both/10	DPD
14	M/31	Adrenal	E	E	1695	49	nd	PR	Local/6	DPD
15	F/20	Abdomen	N	N	6790	231	15	PR	Local/9	DPD
16	M/14	Thor-abdom	N	nd	520	189	1	PR	Disseminated/3	DPD
17	F/39	Thor-abdom	E	E	879	nd	nd	PR	Local/11	AWD
18	F/22	RPG	nd	nd	3318	621	60	PD	Local/1	DPD
19	M/32	RPG	E	E	405	nd	1	PR	Both/11	DPD
20	M/52	RPG	E	nd	625	45	1	PR	Both/24	AWD
21	M/49	RPG	E	N	1072	77	1	PR	Local/11	DPD
22	F/31	RPG	N	N	2445	151	50	CR	Local/13	AWD

M, male; F, female; N, normal; RPG, retroperitoneal ganglia; E, elevated; nd, not done; DPD, dead progressive disease; ADF, alive disease free; AWD, alive with disease.

### High-risk group

Forty-four of 48 patients underwent surgery at onset, which consisted of complete tumor resection in one case (stage 3 for infiltrated contralateral lymph nodes), partial resection in four and biopsy in 39. After induction therapy, three patients were in complete remission, 29 had achieved partial response and 11 minor response. Of the five remaining patients, two experienced early tumor progression and died (Table 3), one died of chemotherapy-related toxicity, one was withdrawn from the study and one could not be evaluated. Twenty-eight patients subsequently underwent surgery, which resulted in complete tumor resection in 11, partial resection in 13 and a biopsy in only four. At this time, 15 patients were not operated on because they were either disease free (three cases), or still inoperable (12 cases). No information about surgery was obtained regarding one patient.

In conclusion, after induction chemotherapy and surgery (if performed), of 43 evaluable patients 14 were in complete remission, and 24 in partial remission, while five had achieved only minor response. Of the 14 patients in complete remission, 10 received no further therapy (four relapsed) and four received additional chemotherapy (two relapsed). Of the 24 patients who had achieved partial response, one died shortly after surgery (due to an electrolyte imbalance), nine received no further therapy (six relapsed), three received additional chemotherapy (two relapsed), two received chemotherapy associated with radiotherapy (one relapsed) and nine were treated with therapeutic doses of MIBG (one relapsed). Of the five patients who had achieved minor response, two received no further therapy and both of them underwent tumor progression, one received chemotherapy and relapsed, and two received therapeutic doses of MIBG (one relapsed). The patient whose surgery information is missing received high-dose chemotherapy and is still alive.

Overall, 23 of 48 patients are alive progression free at 8–87 months (median 49 months). In 22 patients the tumors progressed or relapsed either locally (11 cases), or in distant sites (four cases), or in both (seven cases). Of these 22 patients, two are still alive in second complete remission, five are alive disease free and 15 have died from their disease. Lastly, two patients died of toxicity (chemotherapy-related and surgery-related in one case each) and one was withdrawn from the study during the induction phase.

### Analysis of survival

The estimated cumulative probabilities of OS and event-free survival at 5 years from diagnosis for the entire series of 95 patients are 75 and 60%, respectively. Table 4 refers to the univariate analysis according to the main prognostic factors. Low-risk patients had an OS of 91% compared with 60% of those with high-risk characteristics (Figure 1). OS was significantly better in the 35 infants compared with the 60 children  $\geq 1$  year of age (91 versus 65%;  $P = 0.02$ ). As for tumor markers, normal VMA urinary excretion, high LDH, ferritin and

NSE plasma levels, and amplified *MYCN* gene were all associated with poorer survival. In multivariate analysis, the association between survival and risk group is confirmed ( $P = 0.023$ ) only when the *MYCN* gene copy number is excluded from the model. However, the model including both the risk group and *MYCN* gene amplification showed that the interaction between these two factors had a significant effect ( $P = 0.002$ ), thus demonstrating that the presence of *MYCN* gene amplification increases mortality more significantly in low-risk patients than in the high-risk ones.

## Discussion

The term neuroblastoma encompasses a variety of clinical patterns ranging from the localized resectable tumor (20% of cases) [1, 2, 13], often curable by surgery alone [14], to the widespread tumor (about half of the cases), for which the prognosis remains poor [3, 4]. Predictably, an unresectable neuroblastoma without metastases bears an intermediate prognosis [15]. In the 1980s, less than half of patients with unresectable neuroblastoma treated with a combination of vincristine and cyclophosphamide were curable [13, 16]. Subsequently, by applying an intensive multiple alkylating agent chemotherapy [15, 17] or alternating the combinations of cisplatin–teniposide and cyclophosphamide–doxorubicin [5], the event-free survival of these patients has increased to 60% and above, albeit in small series of patients.

There are few large studies related to unresectable neuroblastoma. In 1993, Garaventa et al. [7] reported on 145 such patients, of whom 77 were treated early with a standard-dose protocol, and 68 later on with a high-dose one. Progression-free survival at 5 years was 50% for the entire series but was significantly better for those treated with the high-dose protocol (59 versus 43%). Infants and children with non-abdominal tumors had a better outcome compared with children  $>1$  year of age with abdominal disease (70 versus 30%). Surprisingly, high-dose therapy did improve progression-free survival in poor-risk patients, but not in good-risk ones. In 1998 Rubie et al. [6] published the French experience on 130 such patients treated with two cycles of carboplatin–etoposide followed by two cycles of vincristine–doxorubicin–cyclophosphamide (CADO regimen). The 5-year event-free survival of the overall population was 78%. *MYCN* oncogene amplification was the most powerful predictor of poor outcome. In fact, children with unresectable neuroblastoma and no *MYCN* amplification fared as well as children with resectable tumors (event-free survivals of 89 and 91%, respectively). In 1998 Matthay et al. [18] reported the Children's Cancer Group's experience on 228 stage 3 patients stratified for risk factors (age, *MYCN* gene copy number, histology type and ferritin levels). Patients with one or more risk factors were treated with more intensive therapy. The event-free survival at 4 years was 100% for patients with no risk factors, 90% for infants with one or more risk factors, and 50% for patients  $>1$  year with one or more risk

**Table 4.** Prognostic factors for 5-year OS

	Patient no.	Survival (%)	Univariate analysis	
			Hazard ratio (95% CI)	<i>P</i> value
All patients	95	75	–	–
Risk group				
Low	47	91	1 (ref.)	0.002
High	48	60	4.73 (1.59–14.1)	
Age at diagnosis				
<1 year	35	91	1 (ref.)	0.020
≥1 year	60	65	3.85 (1.13–13.1)	
Site of primary				
Abdomen	72	72	1 (ref.)	0.295
Other	23	86	0.53 (0.15–1.79)	
VMA (three missing)				
≤2.5 SD	24	56	1 (ref.)	0.007
>2.5 SD	68	83	0.31 (0.12–0.76)	
HVA (14 missing)				
<2.5 SD	20	66	1 (ref.)	0.308
≥2.5 SD	61	79	0.60 (0.22–1.62)	
LDH (three missing)				
<1000 UI/l	59	87	1 (ref.)	0.007
≥1000 UI/l	33	59	3.35 (1.32–8.50)	
Ferritin (15 missing)				
<150 mg/ml	56	83	1 (ref.)	0.011
≥150 mg/ml	24	55	3.03 (1.23–7.48)	
NSE (30 missing)				
<100 mg/ml	42	87	1 (ref.)	<0.001
≥100 mg/ml	23	47	5.24 (1.82–15.1)	
<i>MYCN</i> gene (15 missing)				
<3 copies	68	86	1 (ref.)	<0.001
≥3 copies	12	10	12.8 (4.93–33.4)	

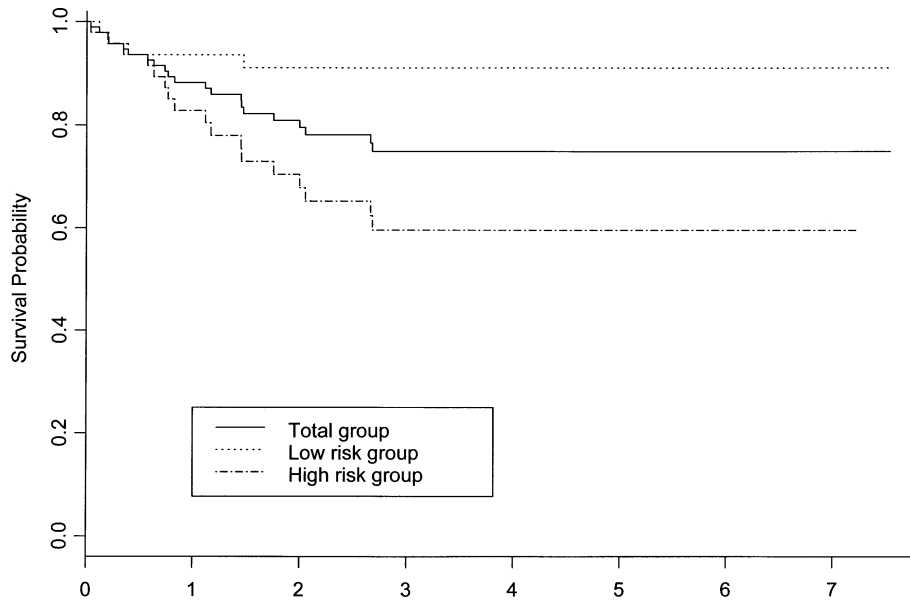
CI, confidence interval; ref, reference group.

factors. Only *MYCN* copy number and age were independent factors in the multivariate analysis. The remarkable results of this and the previous study suggest that the biological characteristics of the tumor play a crucial role in the outcome of stage 3 neuroblastoma, and should be used as a guide for the therapeutic choices.

The present study was designed on the basis of our previous experience [7]. Accordingly, for low-risk patients (who fared well regardless of the intensity of treatment) a standard-dose and therefore scarcely toxic chemotherapy protocol was considered adequate, whereas high-risk patients were thought to possibly benefit from a more aggressive regimen. Somewhat disappointingly, the overall complete response rate thus obtained was slightly lower than in the previous study (45 versus 50%), while the rate of radical tumor resection was

comparable (20 versus 19% of cases). Stricter criteria for defining response to chemotherapy and evaluating the post-operative residual tumor may account in part for the lack of improvement of these two endpoints. In particular, the low rate of surgical success can be accounted for by the rather cautious guidelines given by Italian surgeons, which have resulted in a remarkably low number of surgery-related fatal events.

Although the above figures fail to show an improvement compared with the previous study [7], the overall results of this study do appear superior. The 5-year OS rate of the present series has in fact increased from 55 to 75%, and the event-free survival rate has increased from 50 to 60%. Improvement has occurred in both patient groups. OS in low-risk patients, all of whom avoided aggressive therapy,



**Figure 1.** OS of children with unresectable neuroblastoma ( $n = 95$ ) is 75%. OS of children in low-risk group ( $n = 47$ ) is 91%; OS in high-risk group ( $n = 48$ ) is 60%.

increased from 70 to 91%, and in high-risk patients from 30 to 60%. Therefore, we clearly confirm that infants and children with non-abdominal tumors have an excellent chance of being cured without being exposed to risky chemotherapy. Further reduction of treatment could possibly be applied to these patients.

The improvement that occurred in children with high-risk characteristics is more difficult to explain. Perhaps the more meticulous extensive procedures applied in the present study have avoided enrolling some stage 4 patients with minimal tumor spread. In addition, the somewhat stricter criteria used to evaluate response have, on the one hand, decreased the number of complete remissions, while on the other implied longer and more intense treatment for partial response patients. The availability of carboplatin, which now substitutes the more toxic cisplatin, may also have contributed favorably [6, 19]. Finally, metabolic radiotherapy administered to a number of patients with macroscopic residual tumor may have had an eradicating effect [20].

Most treatment failures (23 of 27) occurred at the primary tumor site, usually within the first 2 years after diagnosis. Out of 26 patients who underwent delayed radical surgery only three relapsed (one local, two both locally and in distant sites) while more than one-third (26 of 69) of patients failing a delayed radical surgery progressed. Interestingly, the characteristics at diagnosis were similar, but none of the 21 evaluated patients who underwent radical surgery had amplification of *MYCN* gene. Although no further therapy was administered to patients in complete remission, this probably did not lead to an increase in relapses (nine of 42 patients). Patients with residual tumors after surgery had a much higher risk of failure

(18 of 48), except for the 16 who received radiometabolic therapy, of whom only two relapsed.

However, after induction therapy and delayed surgery, patients were treated heterogeneously and the role that treatment played is unclear. Only a homogeneous and prospective study can identify the impact on survival of residual tumors and further treatment.

In agreement with others [18, 21], we found that *MYCN* gene amplification acted as a strong predictor of failure. In fact, nine of 12 patients with this anomaly relapsed and only one of them is currently alive. However, *MYCN* amplification occurs in ~20% of cases and therefore identifies only a proportion of patients destined to have an unfavorable clinical course. Better knowledge of the biological characteristics predisposing patients to treatment failure might help in designing more effective therapies for unresectable neuroblastoma.

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