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# Changes over three decades in outcome and the prognostic influence of age-at-diagnosis in young patients with neuroblastoma: A report from the International Neuroblastoma Risk Group Project

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ABSTRACT

*Purpose:* Increasing age has been an adverse risk factor in children with neuroblastoma (NB) since the 1970's, with a 12-month age-at-diagnosis cut-off for treatment stratification. Over the last 30 years, treatment intensity for children >12 months with advanced-stage disease has increased; to investigate if this strategy has improved outcome and/or reduced the prognostic influence of age, we analysed the International Neuroblastoma Risk Group (INRG) database.

*Patients and methods:* Data from 11,037 children with NB (1974–2002) from Australia, Europe, Japan, North America. Cox modelling of event-free survival (EFS) tested if the era and prognostic significance of age-of-diagnosis, adjusted for bone marrow (BM) metastases and *MYCN* status, effects on outcome had changed.

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*Results:* Outcome improved over time: 3-year EFS 46% (1974–1989) and 71% (1997–2002). The risk for those >18 months against ⩽12 decreased: hazard ratio (HR); 4.61 and 3.94. For age 13–18 months, EFS increased from 42% to 77%. Outcome was worse if: >18 months (HR 4.47); BM metastases (HR 4.00); and *MYCN* amplified (HR 3.97). For 1997–2002, the EFS for >18 months with BM involvement and *MYCN* amplification was 18%, but 89% for 0–12 months with neither BM involvement nor *MYCN* amplification.

*Conclusions:* There is clear evidence for improving outcomes for children with NB over calendar time. The adverse influence of increasing age-at-diagnosis has declined but it remains a powerful indicator of unfavourable prognosis. These results support the age-of-diagnosis cut-off of greater than 18 months as a risk criterion in the INRG classification system.



## 1. Introduction

Numerous prognostic clinical and biological factors have been identified in neuroblastoma (NB). Age >12 months and widely disseminated disease were shown to be associated with poor outcome more than 40 years ago.[1,2] Amplification of the *MYCN* oncogene, genetic aberrations of chromosomes 1p, 11q, and 17q, and specific histologic features of the tumour are also associated with poor outcome.[3–8] Combinations of prognostic variables are now routinely used for risk-group assignment and for treatment stratification with significantly intensified regimens for those with high risk disease. If this is an effective strategy, outcome for children with NB should have increased over time while the prognostic influence of variables ascertained at diagnosis should decrease.

To evaluate the influence of these variables on outcome, the International Neuroblastoma Risk Group (INRG) established a database of 11,037 children with NB diagnosed between 1974 and 2002 by Australian, European, Japanese, and North American groups in order to develop a consensus approach to pre-treatment risk stratification. Because treatment regimens have changed substantially over the years, the resulting INRG classification system[9] was based on data from the more recent 8800 patients diagnosed between 1990 and 2002. The analysis identified sixteen risk groups, and age-at-diagnosis played an important role in group identification. The groups were amalgamated into very-low-, low-, intermediate- and high-risk categories based on projected event-free survival rates.

This analysis goes beyond the creation of the INRG classification system[9] as here we examine the changing influence of important prognostic indicators over the whole three decades. The purpose of this paper is to establish the magnitude of any changes and to examine in greater detail the influence of age-at-diagnosis; as a dichotomy at >18 months plays a pivotal role in determining greater risk in the INRG classification.

## 2. Patients and methods

### 2.1. INRG database

Data were collected on patients enrolled on the Children's Oncology Group, German Gesellschaft für Pädiatrische Onkologie und Hämatologie, Japanese Advanced Neuroblastoma Study Group, Japanese Infantile Neuroblastoma Co-operative Study Group, and International Society of Paediatric Oncology Europe Neuroblastoma Group trials. Enrollment cut-off of 2002 was chosen to allow at least two years follow-up at the 2004 data freeze. Eligibility included: confirmed diagnosis of NB or ganglioneuroblastoma (GNB); age ⩽21 years; diagnosis 1974–2002; informed consent. In addition to outcome data, information on 35 potential risk factors was requested although only age-at-diagnosis, the presence of bone marrow (BM) metastases and *MYCN* status are considered in detail here.

### 2.2. Era

In recognition of treatment changes that occurred from 1974 to 2002, the data have been divided into three analytic periods: those diagnosed between 1974–1989, 1990–1996 and 1997–2002. The years defining the era were selected based on major changes in therapeutic strategy. During 1974–1989, multi-agent chemotherapy regimens were introduced, though surgery and radiation therapy remained key modalities. During 1990–1996, the use of risk-based regimens became widespread, and therapy was intensified in patients at greater risk of relapse. Some patients with high-risk disease received stem cell transplants. After 1996, almost all high-risk patients underwent stem cell transplantation. In addition, the use of 13-*cis*-retinoic acid following transplant became widely accepted, and reductions of chemotherapy took place for low- and intermediate-risk patients.

### 2.3. Statistical methods

Event-free survival (EFS) is defined as the period from diagnosis to the first event: relapse, progression, secondary malignancy or death. Patients who experienced no event are censored at the date of last follow-up. Overall survival (OS) is calculated from diagnosis to death, while patients still alive are censored. EFS and OS curves were calculated using the Kaplan–Meier technique. The Cox proportional hazards model was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CI).[10,11] The HR quantifies the increased risk of an event for one group of patients in comparison to another.

### 2.4. Modelling strategy

Univariate Cox regression models, for example for age-at-diagnosis alone, and multivariable models, for example for age and *MYCN* amplification status, have been constructed and compared. The magnitude of the effect of age on outcome is modified by the influence of other variables, particularly the era of initial diagnosis and the therapeutic approach at that time, so different modelling strategies were adopted. Some strategies are dictated by the way age was used 'clinically' to define risk groups.

### 2.5. Justification for modelling with the presence of bone marrow metastases instead of INSS stage

To test the effect of age in a multivariable model adjusting for the extent of disease, one would typically use INSS stage[12,13] for the adjustment. However, the INSS system uses age in the definition of 4s disease; thus, INSS stage and age are confounded. Results below support the use of BM metastases as more highly prognostic than any other site of metastases. As a consequence, the extent of disease is characterised by the presence of BM metastases instead of INSS stage.

### 2.6. Missing values

Age-at-diagnosis was available on all patients; however, serum ferritin, for example, was available on only 1.8% of patients from 1974 to 1989. Because we wish to examine trends over the whole calendar period, we considered only variables that are available in Era I and are of major prognostic importance. In addition an 'Unknown' category (a mixture of patients with and without the attribute) was created for each variable. This enabled, for example, the inclusion *MYCN* status in our models as the 'Unknown' survival curve group in each Era take a central position between the amplified and not amplified groups. This approach allows the modelling process to retain the same numbers of patients irrespective of which variables are included in the Cox model.

### 2.7. Calculations

Calculations were made using Stata version 10 (Stata Corp., 2007).[14]

## 3. Results

### 3.1. Era

From 11,037 patients, 4266 (39%) experienced an event, and 3627 (33%) died (Table 1). Although we focus on era, age, BM metastases and *MYCN* status on outcome, the characterisation of patients by INSS stage, other sites of metastases and initial treatment is presented. The overall 3-year EFS and OS rates were 62% and 70% (Fig. 1). The long-term EFS curve plateaus beyond 5 years at approximately 60%. EFS improved from 43% (Era I: 1974–1989) to 60% (Era II), to 68% (Era III: 1997–2002). Those diagnosed in Era III had one third of the event rate compared to those diagnosed in Era I (HR = 0.35; 95% CI: 0.32–0.38) (Fig. 1, Table 1). There was considerable variation in outcome within each Era, highly dependent on patient and tumour features.

### 3.2. Age-at-diagnosis

The age-at-diagnosis distribution (Fig. 2) is markedly skewed to the younger age groups. Therefore, for the first 2 years of life, EFS and OS analyses were performed on cohorts of patients divided into 3-month (91-day) intervals, whereas 6-month (182-day) intervals were used thereafter. The percentage of patients with an event increased from 15% in the youngest to over 60% in the oldest (Table 2). Further when compared to infants less than 3 months, HRs increase with age up to 911 days (30 months) and then plateau at approximately 5.0. In addition, the corresponding age-specific HRs (fitted by separate Cox models within each era) consistently decrease over time. For example, within Era I, the risk is 2.28 times greater for children with age-of-diagnosis 274–364 days compared to those of 0–91 days. For children of 274–364 days, the risk decreases over time (HRs: 2.28, 1.36, and 0.98 by era). Nevertheless, even in Era III, age remains a strong predictor of adverse outcome in patients >22 months, with HRs close to 4. Despite a gradually increasing risk with increasing age-at-diagnosis beyond 22 months, there is no obvious cut-point for categorisation into low- and high-risk groups.

Using the three broader age categories beyond 12 and 18 months, which correspond to the previous and newly recommended categories for higher risk children suggested by INSS and INRG classification system, respectively (Table 2), shows an increasing HR with age but now more smoothly because of the merged categories. The EFS (Fig. 3) shows a clear decline. There remains a suggestion of a weakening effect of age over the Era with, in general, HRs closer to unity but there remain a clear indication of declining 3-year EFS from 83.82%, through 68.35% to 43.28% as patient age-at-diagnosis increases. The outcome for those of 13–18 months of age has got progressively closer to that of the youngest patients over calendar time.

### 3.3. Bone marrow metastases

In single variable Cox models for EFS, the presence of BM metastases is more highly predictive of an event than any other metastatic site, with a HR = 1.89 for the unknown and 4.00 (CI: 3.76–4.26, $p < 0.001$) for those with involvement. The corresponding HRs for OS are 2.53 and 5.19 (Table 1). The long-term EFS is approximately 75% without BM involvement, 50% when the marrow status is unknown, and only 25% in those with confirmed BM involvement (Fig. 4). There is little relative change in the adverse prognosis associated with BM involvement over the three era, with successive HRs of 3.66, 4.25 and 3.16. Nevertheless, even in patients with BM metastases, the 3-year EFS improved from 22% to 35%, to 45%.

### 3.4. MYCN

*MYCN* status is also highly predictive of outcome with the HRs for amplified tumours 3.97 (CI: 3.64–4.32, $p < 0.001$) for EFS and 5.31 (4.84–5.82, $p < 0.001$) for OS (Table 1). Those unknown

**Table 1 – Variables by Era of diagnosis for NB patients ⩽21 years of age.**

| | | Era I (%) 1974–1989 | Era II (%) 1990–1996 | Era III (%) 1997–2002 | All era (%) | | |
|---|---|---|---|---|---|---|---|
| Number | *n* | 2207 | 5035 | 3795 | 11037 | | |
| Age (y) | Median | 2.14 | 1.41 | 1.16 | 1.44 | | |
| | Range | 0–20.0 | 0–19.5 | 0–20.9 | 0–20.9 | | |
| Age (*m*) | 0–12 | 597 (27) | 2003 (40) | 1748 (46) | 4348 (39) | | |
| | 13–18 | 235 (11) | 585 (12) | 457 (12) | 1277 (12) | | |
| | 19–24 | 218 (10) | 411 (8) | 296 (8) | 925 (8) | | |
| | 25+ | 1157 (52) | 2036 (40) | 1294 (34) | 4487 (41) | | |
| INSS | I | 17 (1) | 777 (15) | 963 (25) | 1757 (16) | | |
| | IIa | 22 (1) | 278 (6) | 276 (7) | 576 (5) | | |
| | IIb | 11 (0) | 245 (5) | 285 (8) | 541 (4) | | |
| | III | 93 (4) | 828 (16) | 660 (17) | 1581 (15) | | |
| | IV | 319 (14) | 1981 (39) | 1261 (33) | 3561 (32) | | |
| | IVs | 10 (0) | 348 (7) | 291 (8) | 649 (6) | | |
| | Unknown | 1735 (79) | 578 (11) | 59 (2) | 2372 (22) | | |
| Metastases | | | | | | Hazard ratio (HR) | |
| | | | | | | EFS | OS |
| Bone marrow | No | 1108 (50) | 3186 (63) | 2707 (71) | 7001 (64) | 1 | 1 |
| | Unknown | 84 (4) | 126 (3) | 315 (8) | 525 (4) | 1.89 | 2.53 |
| | Yes | 1015 (46) | 1723 (34) | 773 (20) | 3511 (32) | 4.00 | 5.19 |
| Bone | No | 1276 (58) | 3587 (71) | 2911 (77) | 7774 (71) | 1 | 1 |
| | Unknown | 109 (5) | 141 (3) | 314 (8) | 564 (5) | 2.14 | 2.80 |
| | Yes | 822 (37) | 1307 (26) | 570 (15) | 2699 (24) | 3.70 | 4.66 |
| Distant lymph nodes | No | 1481 (67) | 3943 (78) | 3181 (84) | 8605 (78) | 1 | 1 |
| | Unknown | 128 (6) | 142 (3) | 345 (9) | 615 (5) | 1.33 | 1.54 |
| | Yes | 598 (27) | 950 (19) | 269 (7) | 1817 (17) | 2.11 | 2.48 |
| Liver | No | 1728 (78) | 4216 (84) | 3166 (83) | 9110 (83) | 1 | 1 |
| | Unknown | 129 (6) | 130 (3) | 307 (8) | 566 (5) | 1.49 | 1.76 |
| | Yes | 350 (3) | 689 (14) | 322 (8) | 1361 (12) | 1.34 | 1.43 |
| Skin | No | 1134 (16) | 4361 (87) | 3325 (88) | 8820 (80) | 1 | 1 |
| | Unknown | 1049 (48) | 578 (11) | 410 (11) | 2037 (18) | 1.90 | 2.36 |
| | Yes | 24 (1) | 96 (2) | 60 (2) | 180 (2) | 0.93 | 0.83 |
| Lung | No | 1144 (52) | 4271 (85) | 3273 (86) | 8688 (79) | 1 | 1 |
| | Unknown | 1049 (48) | 702 (14) | 480 (13) | 2231 (20) | 1.77 | 2.18 |
| | Yes | 14 (1) | 62 (1) | 42 (1) | 118 (1) | 3.05 | 3.58 |
| CNS | No | 1124 (51) | 4279 (85) | 3281 (86) | 8684 (79) | 1 | 1 |
| | Unknown | 1049 (48) | 702 (14) | 480 (13) | 2231 (20) | 1.77 | 2.18 |
| | Yes | 34 (2) | 54 (1) | 34 (1) | 122 (1) | 2.65 | 3.12 |
| Other | No | 1590 (72) | 4149 (82) | 3223 (85) | 8962 (81) | 1 | 1 |
| | Unknown | 157 (7) | 136 (3) | 357 (9) | 650 (6) | 1.68 | 2.00 |
| | Yes | 460 (21) | 750 (15) | 215 (6) | 1425 (13) | 2.46 | 2.81 |
| *MYCN* | Not amplified | 233 (11) | 3035 (60) | 2910 (77) | 6178 (56) | 1 | 1 |
| | Unknown | 1901 (86) | 1357 (27) | 373 (10) | 3631 (32) | 2.33 | 3.12 |
| | Amplified | 73 (3) | 643 (13) | 512 (13) | 1228 (12) | 3.97 | 5.31 |
| Therapy | Observation | 24 (1) | 261 (5) | 80 (0) | 365 (3) | | |
| | Surgery alone | 150 (7) | 736 (15) | 1206 (32) | 2092 (19) | | |
| | Chemo + surgery | 653 (30) | 1612 (32) | 631 (17) | 2896 (26) | | |
| | HDT no stem cell | 189 (9) | 364 (7) | 187 (5) | 740 (7) | | |
| | HD unknown Stem cell | 112 (5) | 500 (10) | 293 (8) | 905 (8) | | |
| | HDT with stem cell | 57 (3) | 399 (8) | 446 (12) | 902 (9) | | |
| | Unknown | 1022 (46) | 1163 (23) | 952 (25) | 3137 (28) | | |
| Event | | 1285 | 2013 | 968 | 4266 | | |
| EFS (%) | 3-year | 46.3 | 63.3 | 71.0 | 62.0 | | |
| | 5-year | 43.3 | 60.4 | 68.3 | 59.1 | | |
| | HR | 1 | 0.60 | 0.45 | | | |
| Dead | | 1224 | 1725 | 678 | 3627 | | |
| OS (%) | 3-year | 52.0 | 71.6 | 80.3 | 70.0 | | |
| | 5-year | 47.1 | 66.8 | 76.6 | 65.3 | | |
| | HR | 1 | 0.53 | 0.35 | | | |

*MYCN* status had HRs of 2.33 and 3.12 for EFS and OS. The long-term EFS for children without *MYCN* amplification is approximately 75%, 50% for unknown status and 25% with *MYCN*-amplified tumours (Fig. 5). Little relative change in the adverse prognosis of *MYCN* amplification across era is seen (Table 3), with successive HRs of 2.50 during 1974–1989

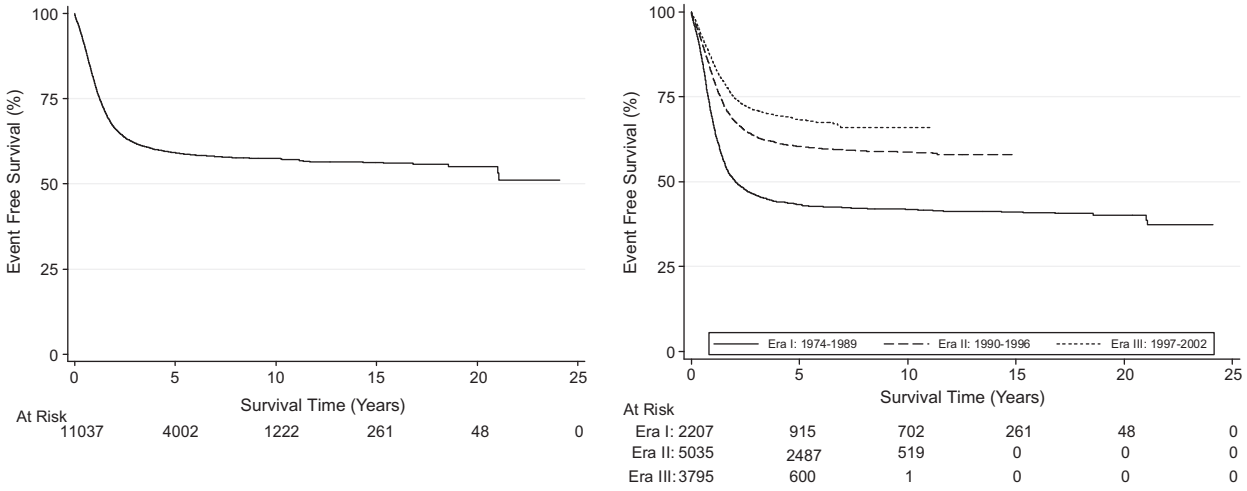


**Fig. 1 – EFS from date of diagnosis of all 11,037 patients and by their respective Era of diagnosis (1974–1989, 1990–1996 and 1997–2002).**

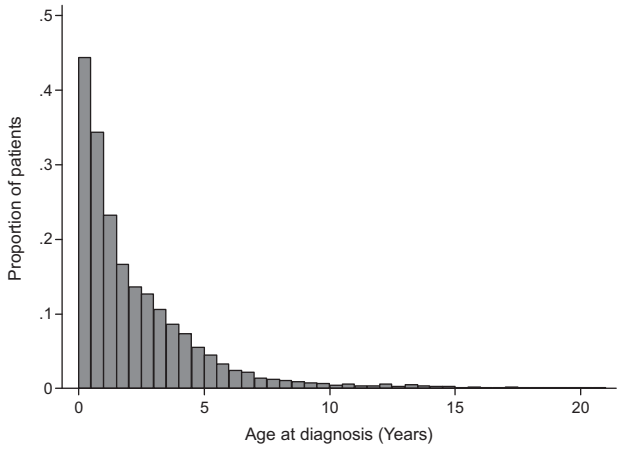


**Fig. 2 – Distribution of age-at-diagnosis in 11,037 cases of neuroblastoma under 21 years of age.**

(an era of many of unknown *MYCN* status), 4.24 and 3.86. Nevertheless, even in patients with *MYCN*-amplification, the 3-year EFS improved from 17% to 28%, to 37% over the decades.

### 3.5. *Age-of-diagnosis adjusted for the presence of bone marrow metastases and MYCN amplification*

Inclusion of BM involvement and *MYCN* status in a multivariable EFS model with age-at-diagnosis weakens the influence of age. For example, the risk for an event for age 25+ months falls from 4.83 in the model with age alone (Table 3, highlighted entries), to 2.95 when adjusted for BM metastases and *MYCN* status. Nevertheless, increasing age is still predictive of adverse prognosis. Adjustment for age and *MYCN* status results in a decrease in the risk associated with the presence of BM metastases, but it remains prognostic of poor outcome in Era III (HR 2.14: 1.87–2.44, $p < 0.001$). Similarly, adjustment for age and the presence of BM metastases results

**Table 2 – Hazard ratios (HRs) for EFS for each Era, and by age (13 and 3 categories) within each Era and all patients combined.**

| Age (*d*) | Age (*m*) | *n* | All events (%) | Hazard ratio (HR) | | | | EFS (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Era I 1974–1989 | Era II 1990–1996 | Era III 1997–2002 | All | 3-year | 5-year |
| 0–91 | 0–3 | 1540 | 237 (15) | 1 | 1 | 1 | 1 | 84.33 | 83.95 |
| 92–182 | 4–6 | 901 | 147 (16) | 1.55 | 1.03 | 0.88 | 1.06 | 83.75 | 82.76 |
| 183–273 | 7–9 | 1170 | 178 (15) | 1.16 | 0.91 | 0.90 | 0.94 | 85.76 | 84.91 |
| 274–364 | 10–12 | 728 | 149 (20) | 2.28 | 1.36 | 0.98 | 1.36 | 79.47 | 78.20 |
| 365–455 | 13–15 | 703 | 183 (26) | 3.98 | 1.39 | 1.34 | 1.78 | 73.03 | 72.84 |
| 456–546 | 16–18 | 575 | 210 (37) | 7.12 | 2.26 | 1.73 | 2.74 | 63.43 | 62.70 |
| 547–637 | 19–21 | 520 | 206 (40) | 4.61 | 2.72 | 2.27 | 3.04 | 58.96 | 57.85 |
| 638–728 | 22–24 | 402 | 206 (51) | 5.38 | 4.19 | 3.99 | 4.41 | 46.96 | 44.11 |
| 729–911 | 25–30 | 750 | 418 (56) | 6.66 | 4.31 | 3.49 | 4.61 | 44.92 | 40.82 |
| 912–1093 | 31–36 | 697 | 434 (62) | 7.07 | 4.58 | 4.47 | 5.14 | 39.49 | 34.78 |
| 1094–1275 | 37–42 | 585 | 362 (62) | 7.47 | 4.42 | 4.10 | 5.12 | 38.96 | 35.22 |
| 1276–1456 | 43–48 | 480 | 305 (64) | 6.40 | 5.08 | 4.12 | 5.11 | 39.77 | 34.10 |
| 1457+ | 49+ | 1986 | 1231 (62) | 5.96 | 4.91 | 3.65 | 4.87 | 41.48 | 34.66 |
| | 0–12 | 4348 | 711 (16) | 1 | 1 | 1 | 1 | 83.82 | 83.04 |
| | 13–18 | 1277 | 397 (31) | 3.94 | 1.70 | 1.63 | 2.11 | 68.35 | 67.92 |
| | 19+ | 5412 | 3158 (58) | 4.61 | 4.29 | 3.94 | 4.47 | 43.28 | 38.32 |
| Total | | 11,037 | 4266 (39) | 2207 | 5035 | 3795 | – | | |

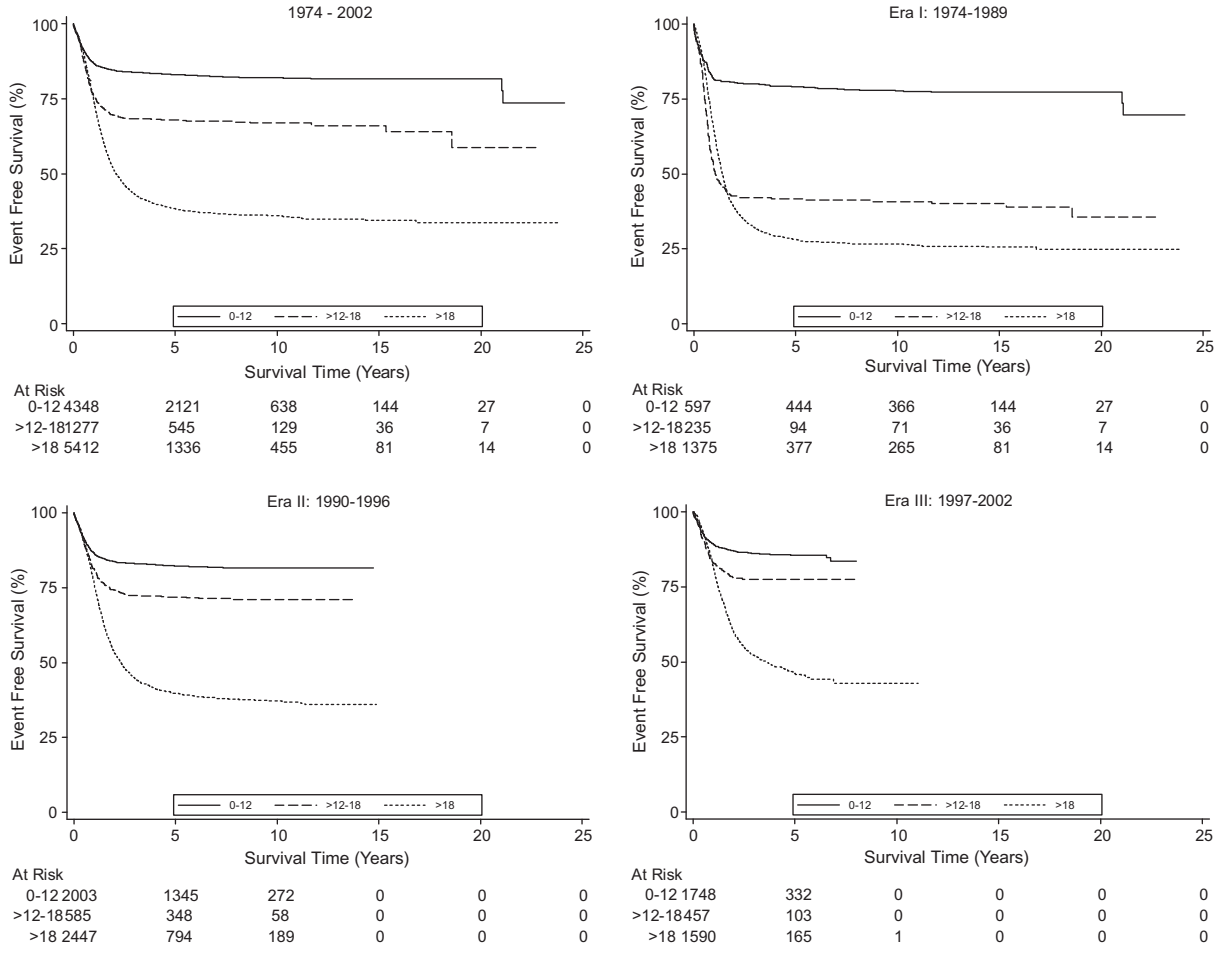


**Fig. 3 – EFS for all 11,037 patients, and within each Era, for the age categories 0–12, 13–18 and 19 or more months.**

in a decrease in the risk associated with *MYCN* amplification compared to the risk if *MYCN* is considered alone. However, amplification remains adversely prognostic in Era III (HR 2.62: 2.35–3.04, $p < 0.001$).

An alternative approach to assessing outcome is to assign a weight of –1, 0, or 1 to each risk factor (age-at-diagnosis, BM metastases, *MYCN* status), where the weight corresponds to the low-, intermediate- and high-risk categories. Using the weights, analyses determined that each risk factor exerted approximately the same level of prognostic influence on outcome (results not shown). On this basis, all possible combinations of low-, intermediate-, or high-risk of age, BM metastases and *MYCN* status create different groups. For each patient, the weights are summed to a score and those with the same score are combined into groups. For example, the weights for patients age-at-diagnosis 0–12 (weight: –1), unknown BM status (0) and amplified *MYCN* (1) sum to 0 (zero). These patients would be combined with others who have a sum of 0, for example, those aged 19+ months (weight: 1), with no BM metastases (–1), and unknown *MYCN* (0). This process results in seven groups with scores ranging from –3 (all factors indicating low-risk) to +3 (high-risk) but then coded 0–6 for convenience. The corresponding EFS curves indicate a worsening prognosis with increasing score, within each era as well as over the entire period (Fig. 6). On this basis, visual inspection of the EFS curves of Era III suggests a possibility of four major risk categories with scores (0, 1), (2), (3), and (4, 5, 6), respectively. This underlines the fact that, despite the considerable improvement in the overall prognosis of young patients with NB, major outcome differences remain.

## 4. Discussion

The International Neuroblastoma Risk Group (INRG) Task Force collated, after extensive consultation and international collaboration, data from 11,037 patients with neuroblastoma (NB) in those less than 21 years of age-at-diagnosis and recruited to clinical studies between 1974 and 2002. With these data INRG have proposed a revised classification system based on the 8800 patients diagnosed between 1990 and 2002.[9,13] The objective of the revised classification was to identify risk groups, ranging from very-good to very-poor prognosis, to enable focus for devising better therapeutic strategies. It is hoped that this will accelerate the improvement in outcome achieved over previous decades for all patients of whatever risk category. A key consideration for this revised classification was whether the age-at-diagnosis cut-off of greater than 12 months should be revised as it had been suggested, from an analysis of 3666 patients, that this might be raised to those who are >18 months.[15] The further data

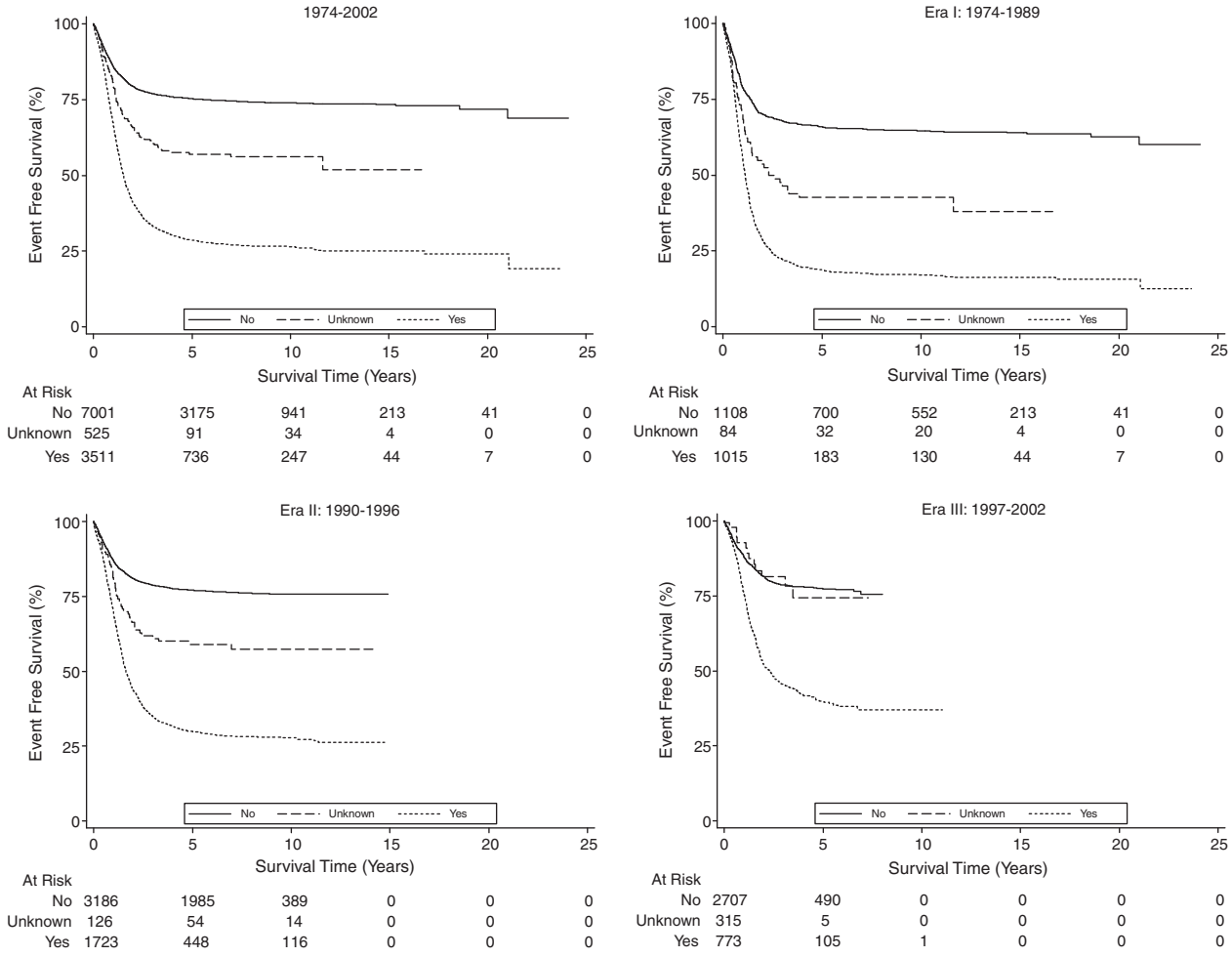


**Fig. 4 – EFS for all 11,037 patients, and within each Era, for the three BM involvement groups.**

available from 1990 to 2002 subset of the INRG database have demonstrated statistical and biological confirmation for the revision. Thus the INRG classification system regards those with age-at-diagnosis >18 months as at higher risk although this is only one of the seven criteria used to determine risk.

Many modifications in treatment and supportive care have occured over time: the three Era (1974–89; 1990–96 and 1997–2002) serve as a surrogate variable encapsulating all factors that have changed. The main focus of this study is to investigate whether the general prognosis for children with NB has improved over time and, in particular, to establish whether age-at-diagnosis still plays a major role in subsequent outcome. It is well established that other features recorded at diagnosis will also be important determinants but to investigate change over the full 28-year period we are constrained to variables that were recorded in the earlier times. This precluded, for example, detailed study of chromosome 11q status, and DNA ploidy but permitted investigation of *MYCN* status and the presence of metastatic disease in the bone marrow. Although stage is a well established clinical prognostic factor in NB, as age is used within the INSS procedure itself to define 4s disease, it could not be considered as a prognostic variable for the purposes of our analysis.

It is recognised that definitions, investigative methods, laboratory techniques and standards of recording are very likely to differ widely over the different international groups each collating information from many individual participating centres on the 11,037 patients concerned. It is also recognised that some subjectivity was utilised in choosing, for example, BM involvement rather than bone metastases for our model although each appeared to be similarly influential on outcome. The former was chosen on statistical grounds as having the fewer missing values, a more even spread in those with and without involvement, and a larger hazard ratio. Further, we were not attempting to develop a full prognostic model for use with future patients (this would need to consider the most recent items now available) but merely to give a broad description of the changes over the era and the influence of age-at-diagnosis using simple statistical models. In full recognition that a substantial part of the data (Era II and III) had been used in developing the INRG classification, we used an entirely different statistical approach, the proportional hazards regression model,[10] to substantiate or otherwise the age-at-diagnosis classification.

We show that the 3-year EFS increased from 46% for patients diagnosed from 1974–1989 to 63% for 1990–1996, to 71% for 1997–2002.

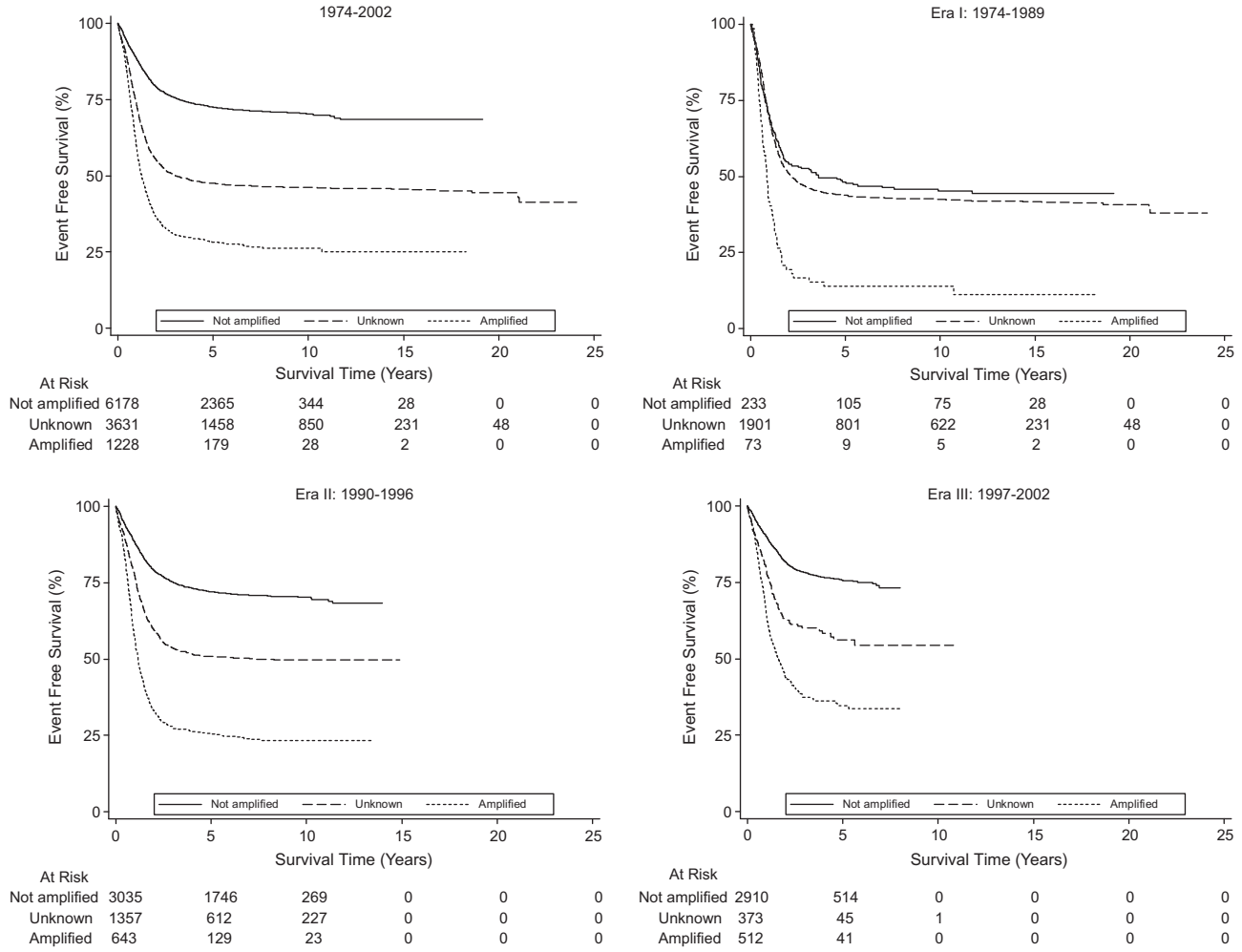


**Fig. 5 – EFS for all 11,037 patients, and within each Era, for the *MYCN* amplification groups.**

**Table 3 – Multivariable estimates of HRs for EFS by age in 6 categories and evidence of BM disease and *MYCN* amplification for each of three era and all times. (Univariate HRs are indicated in parenthesis).**

| | | *n* (%) | All events | Hazard ratio (HR) | | | | EFS (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Era I 1974–1989 | Era II 1990–1996 | Era III 1997–2002 | All era | 3-year | 5-year |
| Age (*m*) | –6 | 2441 (22) | 384 | 1 | 1 | 1 | 1 | 84.11 | 83.51 |
| | 7–12 | 1907(17) | 327 | 1.22 | 0.98 | 0.95 | 1.02 | 83.46 | 82.46 |
| | 13–15 | 703 (6) | 187 | 3.03 | 1.00 | 1.42 | 1.47 | 72.43 | 72.24 |
| | 16–18 | 574 (5) | 210 | 4.52 | 1.97 | 1.51 | 2.34 | 63.38 | 62.65 |
| | 19–24 | 925 (8) | 412 | 3.29 | 2.10 | 2.09 | 2.39 | 53.92 | 52.06 |
| | 25+ | 4487 (41) | 2746 | 3.57 | 2.68 | 2.83 | **2.95** | 41.16 | 35.62 |
| | Univariate HR of 25+ group | | | (5.52) | (4.65) | (4.04) | (**4.83**) | | |
| Bone marrow metastases | No | 7001(63) | 1665 | 1 | 1 | 1 | 1 | 77.00 | 75.36 |
| | Unknown | 525 (5) | 109 | 1.64 | 1.65 | 1.05 | 1.25 | 60.97 | 56.99 |
| | Yes | 3511 (32) | 2492 | 2.81 | 2.86 | 2.14 | 2.68 | 33.34 | 28.62 |
| | Univariate HR of Yes group | | | (3.66) | (4.25) | (3.16) | (4.00) | | |
| *MYCN* amplified | No | 6178 (56) | 1555 | 1 | 1 | 1 | 1 | 75.60 | 72.52 |
| | Unknown | 3631 (33) | 1880 | 0.98 | 1.47 | 1.62 | 1.64 | 50.13 | 47.52 |
| | Yes | 1228 (11) | 831 | 1.88 | 2.77 | 2.62 | 2.58 | 30.80 | 28.28 |
| | Univariate HR of Yes group | | | (2.50) | (4.24) | (3.86) | (3.97) | | |
| | Total | 11037 | 4266 | 2207 | 5035 | 3795 | | | |

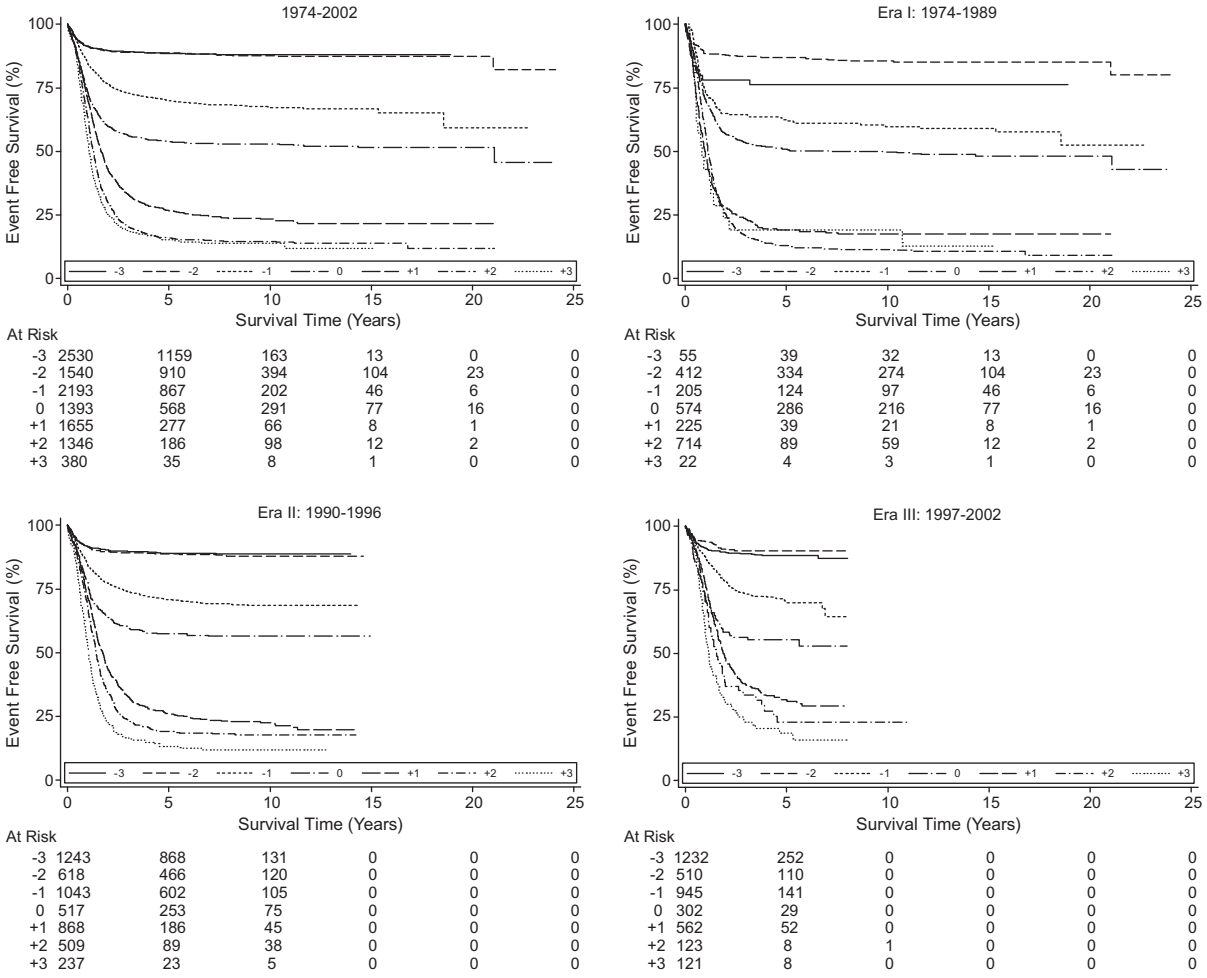


**Fig. 6 – EFS for the seven risk groups defined by age-at-diagnosis (3 groups), together with the presence or absence of metastatic BM involvement and *MYCN* amplification.**

Ignoring all other considerations, age-at-diagnosis appears to have retained an important role over the 28-year period. However, this influence is gradually waning over calendar time with, for example, those of >18 months having a 3-year EFS increasing from 25% in those diagnosed 1974–1989 to 45% in those from 1997–2002 (Fig. 3). Adjusting treatment in those over 12 months to compensate for their relatively poorer prognosis may have contributed to this. It also remains apparent (Table 2) that the risk of relapse increases steadily with age so that no single cut is likely to divide good and poor prognosis unambiguously for all. Thus, there remains some suggestion that those diagnosed between 13 and 18 months remain at higher risk than the youngest children, and that those >18 months are at an even greater risk of relapse. A pragmatic cut beyond 18 months for prognostic purposes for future patients does not seem unreasonable. It is important to note that wherever a cut is made, those close to this (convenient) boundary are at very similar risk. The increasing age risk remains the case, although diminished in magnitude, whether or not the presence or otherwise of metastases in the BM or *MYCN* oncogene status is taken into account. Although only future data can substantiate this, it is likely that strength of increasing age-at-diagnosis as an adverse prognostic variable will continue to decline.

For patients with high-risk disease, treatment strategies have been increasingly intensified over time. Further, the use of myeloablative therapy and stem cell transplants increased following the Children's Cancer Group randomized trial[16] and this too may have contributed to both the improved EFS and the decreasing influence of age-at-diagnosis.

Although older age, *MYCN* amplification and the presence of BM metastases were independently predictive of poor outcome, the unfavourable prognostic influence of either one of these is offset by the absence of one or more of the remaining unfavourable factors (Fig. 6).

It has been suggested that age-at-diagnosis may be a proxy for as undescribed tumour-related factor. However, it may be that some current prognostic features, such as *MYCN*-status, should be reconsidered but on a continuous scale rather than categorised (amplified, not amplified) as when considered in this way they may mimic more closely the situation that is apparent with age.

There is strong evidence that the strategy of increasing therapeutic intensity has improved the outcome for children with NB over the 1974–2002 period and that there is a declining influence of the prognostic effect of age-at-diagnosis. Nevertheless the strength of the three most powerful factors, *MYCN* status, BM metastases and age, remains high.

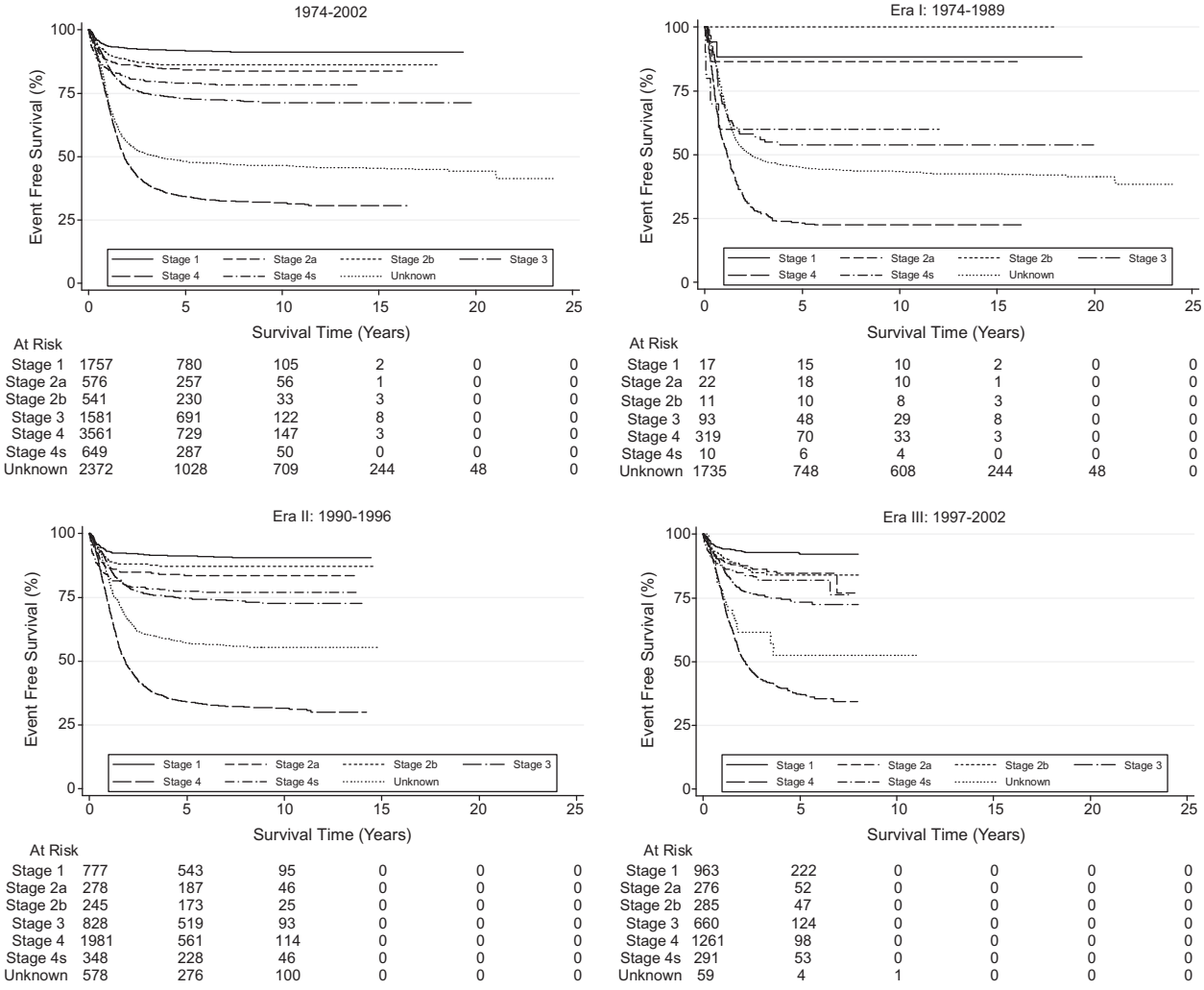


**Fig. A1 – EFS for all 11,037 patients, and within each Era, for each INSS stage.**

## Conflict of interest statement

None declared.

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## Appendix A

See Fig. A1.

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