

SUPPLEMENTARY MATERIAL

Contents

Supplementary information on Methods (pages 2-3)

Definition of False negatives

Definition of Overdiagnosis

Definition of Toxic death

Definition of Incidental diagnosis

Acquisition of Clinical data

Supplementary Table 1 (page 4)

Cumulative incidence of the subcohorts control area, screening area non-participants and participants (all) and screening area participants only according to stage of neuroblastoma in the screening and control areas

Supplementary Table 2 (page 5)

Cumulative incidence of the subcohorts control area, screening area non-participants and participants (all) and screening area participants only according to stage of neuroblastoma in cases aged 12-23 months

Supplementary Table 3 (page 6)

Cumulative mortality 5 years after diagnosis according to stage of neuroblastoma in the screening and control areas during the study birth cohort, in the pre-study, and in the post-study birth cohorts

Supplementary Table 4 (page 7)

Average absolute number of 1382 neuroblastoma patients per birth year according to stage of neuroblastoma in the screening and control areas of the study cohort, in the pre-study, and in the post-study birth cohorts

Supplementary Table 5 (page 8)

Percent of neuroblastoma diagnoses without symptoms at clinical diagnosis per all neuroblastoma diagnoses per birth year

Supplementary Table 6 (page 9)

Average absolute number of tumor- and toxicity-related deaths per birth year in the screening and control areas of the study cohorts, in the pre-study, and in the post-study birth cohorts

Supplementary Figure 1 (page 10)

Kaplan-Meier estimates (EFS and OS) of 1382 neuroblastoma patients in the screening and control areas of the study cohorts, in the pre-study, and in the post-study birth cohorts

Supplementary Figure 2 (page 11-12)

Kaplan-Meier estimates (EFS and OS) of 1375 neuroblastoma patients according to stage in the screening and control areas of the study cohorts, in the pre-study, and in the post-study birth cohorts

Supplementary Figure 3 (page 13-14)

Outcome of patients with neuroblastoma aged 24-71 months at diagnosis comparing patients of the screening cohort and patients of the control cohort. Birth cohorts 1994-99

Supplementary Methods

Definition of False negatives

Patients with a negative screening result and clinical presentation of neuroblastoma up to 71 months were defined as false negative cases.

Definition of Overdiagnosis

Overdiagnosis was defined as the excess incidence rate over the age window investigated (13th to 72nd month) comparing the participants to the control cohort and interpreted as the patients who would not have presented clinically because of spontaneous regression of the tumor, i.e. they were diagnosed unnecessarily. This was determined by subtracting the incidence rate of cases in the screened cohort minus those of the control area. Thus the determined excess is not explained by the early detection in the screening cohort.

Definition of Toxic death

Death by toxicity was calculated only for patients who unequivocally died due to surgical, chemotherapeutic, or other therapies according to clinical reports. Cases in whom the cause remained unclear (tumor- and/or therapy related) were excluded in this analysis.

Definition of Incidental diagnosis

The incidence rate of a diagnosis with a potential for overdiagnosis such as neuroblastoma can also increase through higher awareness and by incidental diagnoses. As a measure of disease awareness, we used the percentage of patients without any reported clinical symptoms. This percentage is typically high in Germany (17) due to the frequent routine health care check-ups in early childhood.

Acquisition of clinical data

Cases were identified by the German Childhood Cancer Registry which receives information on all neuroblastoma cases in Germany and conducts an open-end, long-term vital status follow-up. Clinical data and follow-up data were obtained within the framework of the national neuroblastoma trials covering >97% of all German patients (17). The data lock was July 10, 2020.

Definition of event-free and overall survival

Event-free survival (EFS) was defined as the time from the date of diagnosis until recurrence or progress or until death of any cause or until the last examination. Overall survival (OS) was calculated from the date of diagnosis until death of any cause or until the last examination.

Supplementary Tables

Supplementary Table 1

Cumulative incidence of the subcohorts control area, screening area non-participants and participants (all) and screening area participants only according to stage of neuroblastoma in the screening and control areas¹

	Study birth cohort control area	Study birth cohort screening area	
	1994-99	1994-99 All births	1994-1999 Participants only
Cumulative incidence	Cases per 100,000 births (95%-CI)		
Total	9.3 (8.2-10.3)	13.4 * (12.2-14.6)	15.7 * (14.0-17.4)
Stage 1	1.5 (1.0-1.9)	3.6 * (3.0-4.2)	4.9 * (3.9-5.8)
Stage 2	0.9 (0.5-1.2)	2.3 * (1.8-2.8)	3.4 * (2.6-4.2)
Stage 3	1.8 (1.3-2.3)	2.3 * (1.8-2.8)	2.7 * (2.0-3.4)
Stages 1-3	4.2 (3.5-4.9)	8.3 * (7.3-9.2)	11.0 * (9.5-12.4)
Stage 4S	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Stage 4	5.0 (4.2-5.8)	5.0 (4.3-5.7)	4.4 (3.5-5.3)
Stage missing	0.0 (0.0-0.1)	0.2 (0.0-0.3)	0.3 (0.0-0.5)

¹ Data include cases diagnosed at 12 to 71 months (2nd-6th year) of age

* $P < 0.001$ comparing screening area all patients (detected by screening or clinically) or participants only (detected by screening) to the control area, respectively

Supplementary Table 2

Cumulative incidence of the subcohorts control area, screening area non-participants and participants (all) and screening area participants only according to stage of neuroblastoma in cases aged 12-23 months¹

	Study birth cohort control area	Study birth cohort screening area	
Birth year	1994-1999	1994-1999 All births	1994-1999 Participants only
Cumulative incidence	Cases per 100,000 births (95%-CI)		
Total	3.4 (2.8-4.1)	8.3 * (7.4-9.3)	10.1 * (8.7-11.4)
Stage 1	0.6 (0.3-0.9)	3.0 * (2.4-3.5)	4.2 * (3.3-5.0)
Stage 2	0.5 (0.2-0.8)	2.0 * (1.5-2.4)	2.8 * (2.0-3.5)
Stage 3	0.9 (0.5-1.2)	1.5 * (1.1-1.9)	1.8 * (1.2-1.7)
Stage 1-3	2.0 (1.5-2.5)	6.4 * (5.6-7.2)	8.7 * (7.4-9.9)
Stage 4S	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Stage 4	1.5 (1.0-1.9)	1.7 (1.3-2.2)	1.2 (0.7-1.7)
Stage missing	0.0 (0.0-0.0)	0.2 (0.0-0.3)	0.2 (0.0-0.4)

¹ 12-23 months (2nd year of life) is the typical age of true positive cases detected by the neuroblastoma screening

*P<0.001 comparing all patients in the screening area (detected by screening or clinically) or screening participants only (detected by screening) to the control area, respectively

Supplementary Table 3

Cumulative mortality 5 years after diagnosis according to stage of neuroblastoma in the screening and control areas during the study birth cohort, in the pre-study, and in the post-study birth cohorts¹

	Pre-study birth cohort	Study birth cohort screening area	Study birth cohort control area	Post-study birth cohort	
Birth year	1990-1993	1994-1999	1994-1999	2000-2004	2005-2009
Cumulative 5-year mortality	deaths per 100,000 births (95%-CI)				
Total	3.2 (2.7-3.7)	3.1 * ** (2.5-3.6)	3.4 * (2.7-4.0)	2.9 (2.5-3.4)	2.4 (2.0-2.9)
Stage 1	0.0 (0.0-0.1)	0.2 (0.0-0.3)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Stage 2	0.0 (0.0-0.0)	0.1 (0.0-0.2)	0.0 (0.0-0.1)	0.1 (0.0-0.1)	0.0 (0.0-0.2)
Stage 3	0.4 (0.2-0.5)	0.3 (0.1-0.5)	0.4 (0.2-0.6)	0.3 (0.2-0.5)	0.0 (0.0-0.2)
Stage 1-3	0.4 (0.2-0.6)	0.6 (0.4-0.9)	0.5 (0.2-0.7)	0.4 (0.2-0.5)	0.2 (0.1-0.4)
Stage 4S	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.1)
Stage 4	2.7 (2.2-3.2)	2.4 (1.9-2.9)	2.9 (2.3-3.5)	2.6 (2.1-3.0)	2.2 (1.7-2.6)
Stage missing	0.1 (0.0-0.2)	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.0 (0.0-0.0)	0.0 (0.0-0.1)

¹ Data include cases diagnosed at 12 to 71 months (2nd-6th year) of age

* $P=0.97$ testing the hypothesis that the birth cohort (1994-99, study and control area combined) is not different from all other time cohorts and areas, adjusted for age groups

** $P=0.16$ testing the hypothesis that the study area (1994-99) is not different from all other birth cohorts and areas, adjusted for age groups

Supplementary Table 4. Average absolute number of 1382 neuroblastoma patients per birth year according to stage of neuroblastoma in the screening and control areas of the study cohort, in the pre-study, and in the post-study birth cohorts¹

Birth year	Pre-study birth cohort	Study birth cohort screening area	Study birth cohort control area	Post-study birth cohort		
	1990-1993	1994-1999	1994-1999	2000-2004	2005-2009	2010-2014
	Average number of cases (95%-CI)					
All stages	63.3 (56.3-70.7)	58.7 (53.4-64.2)	31.5 (27.5-35.9)	61.2 (55.2-67.6)	56.4 (50.6-62.6)	51.0 (45.4-56.9)
Stage 1	7.0 (4.8-9.9)	16.3 (13.4-19.7)	5.0 (3.5-7.0)	7.2 (5.1-9.8)	7.4 (5.3-10)	4.6 (3-6.8)
Stage 2	6.5 (4.4-9.3)	10.3 (8-13.1)	2.8 (1.7-4.4)	6.0 (4.1-8.4)	6.8 (4.8-9.3)	8.6 (6.3-11.4)
Stage 3	11.8 (8.8-15.4)	9.8 (7.6-12.5)	6.2 (4.4-8.4)	10.2 (7.7-13.2)	8.6 (6.3-11.4)	8.2 (6-11)
Stage 4S / multilocular ²	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0.4 (0.1-1.3)	1.0 (0.4-2.2)	0.8 (0.3-1.9)
Stage 1-3	25.3 (20.9-30.1)	36.5 (32.5-40.7)	14.0 (11.4-17.0)	23.8 (20.1-28.2)	23.2 (19.5-27.3)	22.0 (18.4-26.0)
Stage 4	38.0 (32.5-44)	22.2 (18.8-26)	17.5 (14.5-20.9)	37.4 (32.6-42.7)	32.6 (28.1-37.6)	28.8 (24.5-33.5)
Stage missing	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)

¹ Data include cases diagnosed at 12 to 71 months (2nd-6th year) of age

² Stage 4S older than 1 year at diagnosis (12-18 months) applied only after 2000 according to modified inclusion criteria

Supplementary Table 5

Percent of neuroblastoma diagnoses without symptoms at clinical diagnosis per all neuroblastoma diagnoses per birth year ¹

Birth year	Pre-study birth cohort	Study birth cohort screening area	Study birth cohort control area	Study birth cohort Screening + control area	Post-study birth cohorts		
	1990-1993	1994-1999	1994-1999	1994-1999	2000-2004	2005-2009	2010-2014
	Percent (95%-CI) of cases per year						
All stages	15.8% (11.7-20.7)	53.4% (48.2-58.6)	15.9% (11.2-21.6)	40.3% (36.2-44.5)	17.0% (13.1-21.5)	21.3% (16.8-26.3)	17.3% (13-22.2)
Stage 1-3	32.7% (24.1-42.2)	74.9% (68.8-80.3)	32.1% (22.9-42.6)	63.0% (57.5-68.3)	35.0% (26.8-44.0)	40.4% (31.7-49.5)	22.4% (15.3-31.0)
Stage 4S	0.0% (0.0-0.0)	0.0% (0.0-0.0)	0.0% (0.0-0.0)	0.0% (0.0-0.0)	50% (6.1-93.9)	20% (2.3-62.9)	50% (12.3-87.7)
Stage 4	4.6% (2.1-8.8)	18.0% (12.2-25.2)	2.9% (0.8-7.4)	11.3% (7.8-15.8)	5.3% (2.8-9.3)	8.0% (4.5-12.9)	12.5% (7.9-18.6)
Stage missing	0.0% (0.0-0.0)	0.0% (0.0-0.0)	0.0% (0.0-0.0)	0.0% (0.0-0.0)	0.0% (0.0-0.0)	0.0% (0.0-0.0)	0.0% (0.0-0.0)

¹ Data include cases diagnosed at 12 to 71 months (2nd-6th year) of age

Supplementary Table 6

Average absolute number of tumor- and toxicity-related deaths per birth year in the screening and control areas of the study cohorts, in the pre-study, and in the post-study birth cohorts¹

	Pre-study birth cohort	Study birth cohort screening area	Study birth cohort control area	Post-study birth cohort		
Birth year	1990-1993	1994-1999	1994-1999	2000-2004	2005-2009	2010-2014 ²
	Average number of deaths (95%-CI)					
All stages						
By tumor	20.5 (16.7-24.9)	13.5 (11.0-16.4)	11.8 (9.4-14.6)	22.6 (19.0-26.5)	17.0 (13.9-20.5)	12.4 (9.7-15.5)
By tumor or toxicity	1.3 (0.5-2.7)	0.5 (0.1-1.3)	0.5 (0.1-1.3)	0.6 (0.2-1.6)	0.8 (0.3-1.9)	0 (0-0)
By toxicity	5.0 (3.2-7.5)	1.8 (1-3.1)	0.7 (0.2-1.6)	1.2 (0.5-2.5)	2.0 (1-3.5)	2.0 (1-3.5)
Stages 1-3						
By tumor	1.3 (0.5-2.7)	2.0 (1.1-3.4)	1.7 (0.9-2.9)	2.8 (1.6-4.5)	1.6 (0.8-3.0)	2 (1.0-3.5)
By tumor or toxicity	0.3 (0.0-1.2)	0.2 (0.0-0.8)	0 (0-0)	0.2 (0.0-0.9)	0 (0-0)	0 (0-0)
By toxicity	1.3 (0.5-2.7)	0.7 (0.2-1.6)	0.0 (0-0)	0.4 (0.1-1.3)	0.2 (0-0.9)	0.2 (0-0.9)

¹ Data include cases diagnosed at 12 to 71 months (2nd-6th year) of age

² Observation period still incomplete. Data lock October 16th, 2020.

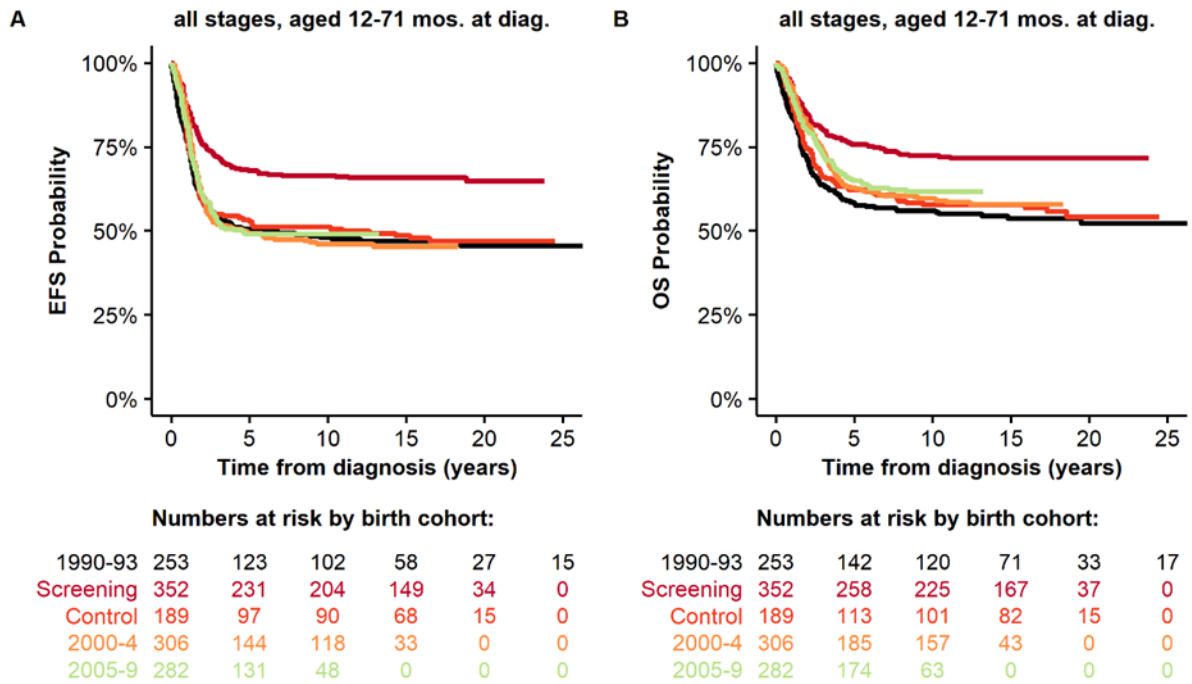
Supplementary Figures

Supplementary Figure 1

Kaplan-Meier estimates (EFS and OS) of 1382 neuroblastoma patients in the screening and control areas of the study cohorts, in the pre-study, and in the post-study birth cohorts¹

A EFS of all stages aged 12-71 months at diagnosis

B OS of all stages aged 12-71 months at diagnosis



Cohort	A	B
	10-y EFS (95%-CI)	10-y OS (95%-CI)
Pre-study 1990-93 (N=253)	48.0% (43.6% - 54.4%)	55.9% (49.7% - 62.1%)
Study Screening area 1994-99 (N=352)	66.5% (64.5% - 71.5%)	72.3% (67.5% - 77.1%)
Study Control area 1994-99 (N=189)	51.1% (43.7% - 58.5%)	57.7% (50.5% - 64.9%)
Post-study:2000-4 (N=306)	56% (40.2% - 61.8%)	59.7% (54.1% - 65.3%)
Post-study 2005-9 (N=282)	49.1% (43.1% - 55.1%)	61.6% (55.6% - 67.6%)

Supplementary Figure 2

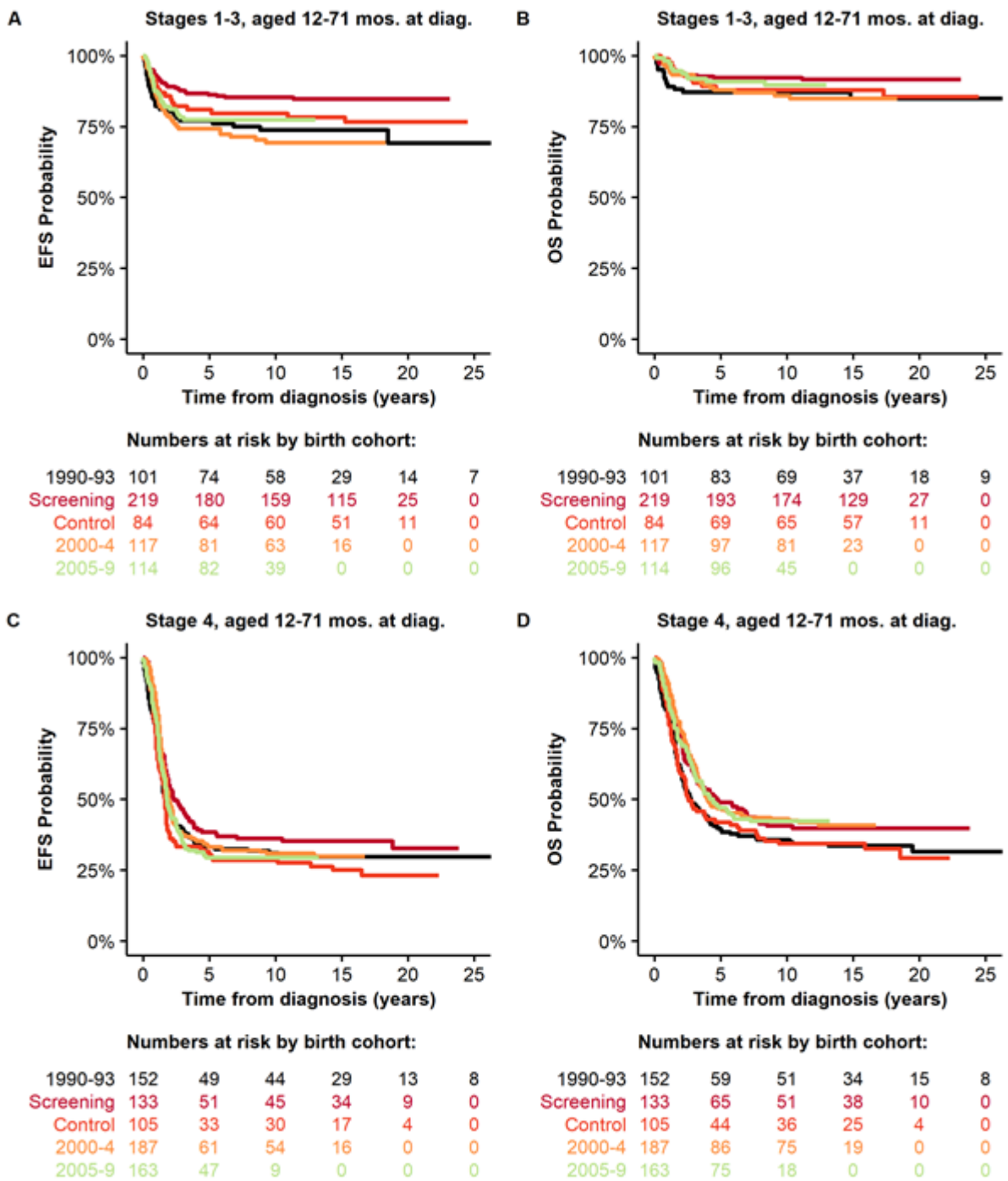
Kaplan-Meier estimates (EFS and OS) of 1375 neuroblastoma patients according to stage in the screening and control areas of the study cohorts, in the pre-study, and in the post-study birth cohorts¹

A EFS of stages 1-3 aged 12-71 months at diagnosis

B OS of stages 1-3 aged 12-71 months at diagnosis

C EFS of stage 4 aged 12-71 months at diagnosis

D OS of stage 4 aged 12-71 months at diagnosis



Cohort	A	B
Stages 1-3	10-y EFS (95%-CI)	10-y OS (95%-CI)
Pre-study 1990-93 (N=101)	73.8% (65.0% - 82.6%)	87.1% (80.5% - 93.7%)
Study screening area 1994-99 (N=219)	85.2% (80.4% - 90.0%)	92.2% (88.6% - 95.8%)
Study control area 1994-99 (N=84)	79.6% (70.8% - 88.4%)	87.8% (80.6% - 95.0%)
Post-study 2000-4 (N=117)	69.3% (60.5% - 78.1%)	85.9% (79.3% - 92.5%)
Post-study 2005-9 (N=114)	77.3% (69.3% - 85.3%)	89.6% (83.6% - 95.6%)
	C	D
Stage 4	10-y EFS (95% CI)	10-y OS (95% CI)
Pre-study 1990-93 (N=152)	31.1% (23.5% - 38.7%)	35.7% (27.9% - 43.5%)
Study screening area 1994-99 (N=133)	36.1% (27.7% - 44.5%)	40.6% (32% - 49.2%)
Study control area 1994-99 (N=105)	28.6% (19.8% - 37.4%)	34.3% (25.1% - 43.5%)
Post-study 2000-4 (N=187)	30.9% (24.1% - 37.7%)	43% (35.8% - 50.2%)
Post-study 2005-9 (N=163)	29.5% (22.3% - 36.7%)	42.2% (34.4% - 50%)

Supplementary Figure 3

Outcome of patients with neuroblastoma aged 24-71 months at diagnosis comparing patients of the screening cohort and patients of the control cohort. Birth cohorts 1994-99.¹

A EFS all patients (N=188)

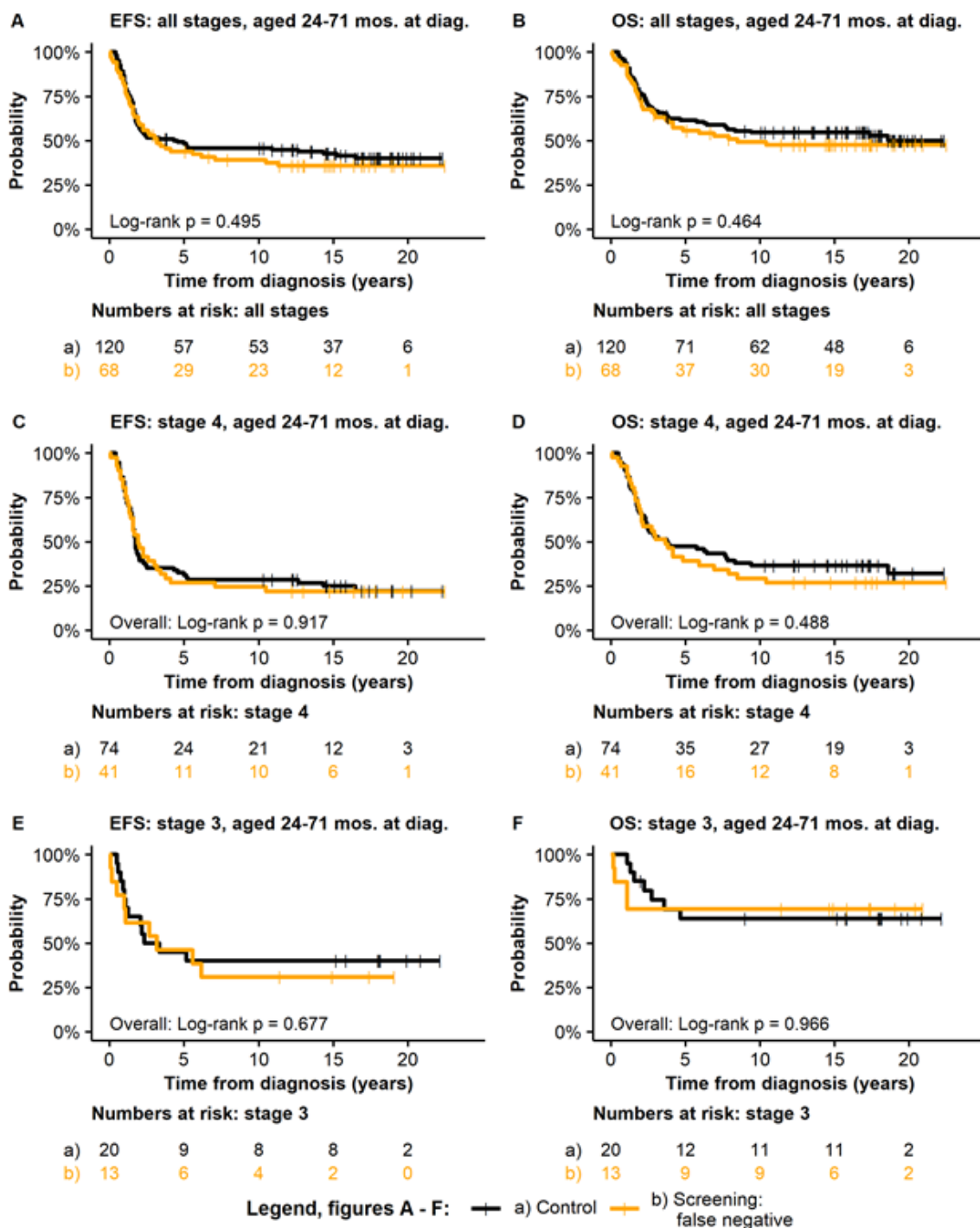
B OS all patients (N=188)

C EFS stage 4 patients (N=115)

D OS stage 4 patients (N=115)

E EFS stage 3 patients (N=33)

F OS stage 3 (N=33)



¹ Patients with true positive screening result are absent due to the screening period

Study birth cohort 1994-99 aged 24 – 71 months at diagnosis			
Figure		A	B
All stages (N=188)		10-y EFS (95%-CI)	10-y OS (95%-CI)
Control area (N=120)		45.7% (37.5% - 55.5%)	54.5% (46.2% - 64.3%)
Screening area (N=68)	True positive (N=0)	-	-
	False negative (N=68)	39.1% (29% - 52.8%)	49.3% (38.6% - 62.9%)
Figure		C	D
Stage 4 (N=115)		10-y EFS (95% CI)	10-y OS (95% CI)
Control area (N=74)		28.4% (14.2% - 41.8%)	36.5% (27.0% - 49.3%)
Screening area (N=41)	True positive (N=0)	-	-
	False negative (N=41)	24.4% (12.4% - 42.0%)	29.3% (18.2% - 47.1%)
Figure		E	F
Stage 3 (N=33)		10-y EFS (95% CI)	10-y OS (95% CI)
Control area (N=20)		40% (23.4% - 68.4%)	63.7% (45.5% - 89.3%)
Screening area (N=13)	Positive (N=0)	-	-
	False negative (N=13)	30.8% (13.6% - 69.5%)	69.2% (48.2% - 99.5%)