Protocol for the Examination of Specimens from Patients with Neuroblastoma

Protocol applies to neuroblastoma and related neuroblastic tumors.

No AJCC/UICC TNM Staging System
The International Neuroblastoma Staging System is recommended

Protocol web posting date: October 2009

Procedures
- Resection
- Biopsy

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Important Note

First priority should always be given to formalin-fixed tissue for morphologic evaluation. Special studies (eg, ploidy analysis, fluorescence in situ hybridization) are critical to the molecular workup of neuroblastoma and require at least 100 mg of viable, snap-frozen tissue as the second priority for workup **(Note A).**

For more information, contact: The Children's Oncology Group Biopathology Center, Phone: (614) 722-2890 or (800) 347-2486.

The College of American Pathologists offers these protocols to assist pathologists in providing clinically useful and relevant information when reporting results of surgical specimen examinations of surgical specimens. The College regards the reporting elements in the “Surgical Pathology Cancer Case Summary (Checklist)” portion of the protocols as essential elements of the pathology report. However, the manner in which these elements are reported is at the discretion of each specific pathologist, taking into account clinician preferences, institutional policies, and individual practice.

The College developed these protocols as an educational tool to assist pathologists in the useful reporting of relevant information. It did not issue the protocols for use in litigation, reimbursement, or other contexts. Nevertheless, the College recognizes that the protocols might be used by hospitals, attorneys, payers, and others. Indeed, effective January 1, 2004, the Commission on Cancer of the American College of Surgeons mandated the use of the checklist elements of the protocols as part of its Cancer Program Standards for Approved Cancer Programs. Therefore, it becomes even more important for pathologists to familiarize themselves with these documents. At the same time, the College cautions that use of the protocols other than for their intended educational purpose may involve additional considerations that are beyond the scope of this document.

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Surgical Pathology Cancer Case Summary (Checklist)

Protocol web posting date: October 2009

NEUROBLASTOMA: Resection, Biopsy

Select a single response unless otherwise indicated.

Specimen
___ Adrenal/periadrenal
___ Retroperitoneal, nonadrenal
___ Thoracic paraspinal
___ Cervical
___ Other (specify): _____
___ Not specified

Procedure (Note B)
___ Resection
___ Incisional biopsy
___ Other (specify): _____
___ Not specified

***Specimen Size**
*Greatest dimension: ___ cm
*Additional dimensions: ___ x ___ cm

***Specimen Weight**
*Specify: ___ g

Specimen Laterality (select all that apply)
___ Right
___ Left
___ Midline
___ Other (specify): _____
___ Not specified

Tumor Size
Greatest dimension: ___ cm
*Additional dimensions: ___ x ___ cm
___ Cannot be assessed (see Comment)

Tumor Weight (if separate from total specimen)
Specify: ___ g
___ Cannot be assessed

* Data elements with asterisks are not required. However, these elements may be clinically important but are not yet validated or regularly used in patient management.

Patient Age
___ Not specified
___ <18 months
___ ≥18 months and <5 years
___ ≥5 years

Histologic Type (select all that apply) (Note C)
___ Neuroblastoma
___ Ganglioneuroblastoma
    ___ Nodular subtype[#] (specify number of nodules: _____)
    ___ Intermixed subtype
___ Ganglioneuroma
___ Indeterminate
___ Cannot be assessed

[#]*Note: For nodular (composite) ganglioneuroblastomas with more than 1 nodule, degree of differentiation and mitotic-karyorrhectic index (MKI) must be given for each nodule. Please indicate the differentiation and MKI for the least favorable nodule in the checklist below. Classification of additional nodules can be described in the Comment.*

Degree of Differentiation (neuroblastic component) (Note D)
___ Undifferentiated
___ Poorly differentiated
___ Differentiating
___ Cannot be assessed
___ Not applicable

Mitotic-Karyorrhectic Index (MKI) (neuroblastic component) (Note E)
___ Low (<100 per 5000 cells; <2%)
___ Intermediate (100-200 per 5000 cells; 2%-4%)
___ High (>200 per 5000 cells; >4%)
___ Indeterminate
___ Cannot be assessed
___ Not applicable

***Tumor Calcification**
*___ Present
*___ Not identified
*___ Cannot be assessed

Treatment History
___ No known presurgical chemotherapy
___ Presurgical chemotherapy given
___ Not specified

International Neuroblastoma Pathology Classification (INPC) (select all that apply)

Note: INPC applies to untreated primary tumors and tumors in metastatic sites provided that there is sufficient material to classify histologically. Bone marrow biopsy is useful only for evaluation of degree of neuroblastic differentiation, but not eligible for MKI determination.

___ **Favorable Histopathology**
- ___ Any age; ganglioneuroma (Schwannian stroma-dominant); maturing or mature
- ___ Any age; ganglioneuroblastoma, intermixed (Schwannian stroma-rich)
- ___ Less than 18 months old; neuroblastoma (Schwannian stroma-poor) or nodular ganglioneuroblastoma; poorly differentiated or differentiating subtypes with low or intermediate mitosis-karyorrhexis index (MKI)
- ___ 18 months up to less than 5 years old; neuroblastoma (Schwannian stroma-poor) or nodular ganglioneuroblastoma; differentiating subtype and low MKI

___ **Unfavorable Histopathology**
- ___ Any age; neuroblastoma (Schwannian stroma-poor) or nodular ganglioneuroblastoma with undifferentiated histology and any MKI
- ___ Less than 18 months old; neuroblastoma (Schwannian stroma-poor) or nodular ganglioneuroblastoma with poorly differentiated or differentiating subtypes with high MKI
- ___ 18 months up to less than 5 years old; neuroblastoma (Schwannian stroma-poor) or nodular ganglioneuroblastoma; poorly differentiated and any MKI, or differentiating and intermediate or high MKI
- ___ Equal to or greater than 5 years old; neuroblastoma (Schwannian stroma-poor) or nodular ganglioneuroblastoma; any subtype and any MKI

___ Not applicable secondary to previous chemotherapy
___ Cannot be determined secondary to insufficient material
___ Indeterminate

Margins
___ Cannot be assessed
___ Margins uninvolved by tumor
___ Margin(s) involved by tumor
Specify margin(s): _____

***Lymph-Vascular Invasion**
*___ Not identified
*___ Present
*___ Indeterminate

Extent of Tumor

Primary Tumor
___ Cannot be assessed
___ Encapsulated
___ Extracapsular extension without adjacent organ involvement
___ Extension into adjacent organs
___ Extension into spinal canal

Regional Lymph Nodes
___ Cannot be assessed
___ Regional lymph node metastasis not identified
___ Regional lymph node metastasis present
Specify site: _____
Number of lymph nodes examined: ___
Number of lymph nodes involved by tumor: ___

Distant Metastasis
___ Cannot be assessed
___ Distant metastasis
*Specify site(s), if known: _____

International Neuroblastoma Staging System (INSS)[#] (Notes F and G)
___ Stage 1
- localized tumor with complete gross excision, with or without microscopic residual disease
- representative ipsilateral nonadherent lymph nodes negative for tumor microscopically (nodes attached to and removed with the primary tumor may be positive)

___ Stage 2A
- localized tumor with incomplete gross excision; representative ipsilateral nonadherent lymph nodes negative for tumor microscopically

___ Stage 2B
- localized tumor with or without complete gross excision with ipsilateral nonadherent lymph nodes positive for tumor; enlarged contralateral lymph nodes must be negative microscopically

___ Stage 3
- unresectable unilateral tumor infiltrating across the midline,[##] with or without regional lymph node involvement
- localized unilateral tumor with contralateral regional lymph node involvement
- midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement

___ Stage 4
- any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs (except as defined for stage 4S[###])

___ Stage 4S
- localized primary tumor (as defined for stage 1, 2A, or 2B), with dissemination limited to skin, liver, and/or bone marrow[###] (limited to infants less than 1 year old)

[#] *Multifocal primary tumors (eg, bilateral adrenal primary tumors) should be staged according to the greatest extent of disease, as defined above, and followed by a subscript "M" (eg, 3_M).*

[##] *The midline is defined as the vertebral column. Tumors originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.*

[###] *Marrow involvement in stage 4S should be minimal (ie, less than 10% of total nucleated cells identified as malignant on bone marrow biopsy or marrow aspirate). More extensive marrow involvement would be considered stage 4. The meta-iodobenzylguanidine (MIBG) scan (if performed) should be negative in the marrow.*

* Data elements with asterisks are not required. However, these elements may be clinically important but are not yet validated or regularly used in patient management.

***Additional Pathologic Findings (Notes H, I, and J)**

**MYCN* Amplification Status
*___ Not assessed
*___ Not amplified
*___ Amplified
*___ Gain
*___ Indeterminate

Note: Results of MYCN amplification information may not be available to the pathologist at the time of the report.

*Other
*Specify: _____

***Comment(s)**

Explanatory Notes

A. Submission of Tissue

Molecular testing is crucial for accurate risk stratification and clinical decision-making. In addition to the tissue taken for histologic examination as described below, the International Neuroblastoma Pathology Committee recommends sampling a neuroblastic surgical specimen for biologic studies as follows:[1]

A minimum of 2 samples (A and B, each 1 x 1 x 1 cm) should be taken, preferably from morphologically different areas. Samples A and B are split into 4 pieces:

1	**2**
3	**4**

A,B 1 Make at least 10 touch preparations (air-dried, unfixed, and, if necessary, stored at –20°C) for fluorescence in situ hybridization (FISH) (*MYCN*, chromosome 1p) and image cytometry

A,B 2 Put in sterile culture medium (for *MYCN*, chromosome 1p, ploidy, cytogenetics, culture and drug sensitivity, etc)

A,B 3,4 Snap-freeze in liquid nitrogen or at –70°C (for molecular biology studies and immunohistochemistry) (also snap-freeze residuum of A,B 1)

The above recommendations are applicable when the entire or a large proportion of the tumor is resected, or when 1 or more large biopsy specimens are available. If the amount of tumor tissue is restricted, morphologic diagnosis is the prime consideration. Imprints (for FISH study of *MYCN*) should always be made from fresh tumor tissue.

If, as a minimum procedure, only core biopsies are performed, they should be multiple (2 to 4, for formalin fixation and snap-freezing), preferably concomitant with fine-needle aspiration specimens for FISH study of *MYCN*. A minimum of 100 mg snap-frozen tissue may be necessary for ploidy study by flow cytometry. Such specimens are usually not sufficient for prognostic evaluation histopathologically.[1]

B. Procedures

Core needle biopsies can obtain sufficient material for special studies and morphologic diagnosis, but sampling problems may limit tumor subtyping or grading, especially in tumors that are heterogeneous (ie, ganglioneuroblastoma, nodular type). Grading can be performed on samples from metastatic sites provided that the specimen is large enough to be representative. When handling an excision specimen sections should be obtained from central and peripheral areas of the tumor according to common guidelines (at least 1 tumor section per centimeter in the longest dimension and sections from all inked surgical margins).[1] All grossly visible nodules or hemorrhagic foci should be individually sampled.

C. Histopathologic Type
It is recommended that the International Neuroblastoma Classification[1,2] described below be used when describing tumor samples.

There are 4 specific categories in this group of tumors:

Neuroblastoma (Schwannian stroma-poor)
Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor)
Ganglioneuroblastoma, intermixed (Schwannian stroma-rich)
Ganglioneuroma (Schwannian stroma-dominant)

Neuroblastoma (Schwannian Stroma-poor) Category
Microscopically, tumors in the neuroblastoma category are composed of neuroblastic cells that form groups or nests separated by delicate, often incomplete stromal septa without or with limited Schwannian proliferation (comprising <50% of the tumor).[1]

Differential Diagnosis
The differential diagnosis of neuroblastoma usually also includes the pediatric small round blue cell tumors: peripheral primitive neuroectodermal tumor (pPNET)/Ewing sarcoma, alveolar rhabdomyosarcoma, Wilms tumor, desmoplastic small round cell tumor, lymphoma, and myeloid leukemia. A cell surface glycoprotein, p30/32 (product of the *MIC2* gene detected by CD99 antibodies), common in peripheral primative neuroectodermal tumor (pPNET)/Ewing sarcoma and lymphomas, usually is negative in neuroblastoma; both neuroblastoma and pPNET are frequently positive for PGP9.5 and NB84. In contrast, tyrosine hydroxylase commonly is positive in neuroblastoma and negative in pPNET/Ewing sarcoma. Muscle-specific markers, such as desmin, myogenin, and MyoD1, are often positive in rhabdomyosarcomas but negative in neuroblastoma; additionally, rhabdomyosarcoma cells often show morphologic evidence of muscle differentiation. Although the blastemal component of a Wilms tumor may mimic neuroblastoma, the former often exhibits WT1 positivity in addition to epithelial and mesenchymal components. Finally, lymphomas usually stain for multiple lineage-specific hematopoietic markers, whereas neuroblastomas are negative for these proteins. Undifferentiated neuroblastoma cells may, on rare occasions, express vimentin.

Electron Microscopy
Ultrastructural studies are still of value in the diagnosis of relatively undifferentiated neuroblastoma, where the diagnosis is not readily evident by light microscopic study or urinary catecholamine study, especially given the variable specificity of immunostaining. Diagnostic criteria include dense core granules of neurosecretory type and cell processes (primitive neurites) containing typically arranged microtubules.

Ganglioneuroblastoma, Nodular (Composite Schwannian Stroma-rich/Stroma-dominant and Stroma-poor) Category[#]
Tumors in the ganglioneuroblastoma, nodular category are composed of multiple clones: one or more nodules of neuroblastic cells set within a background of ganglioneuroblastoma, intermixed, or ganglioneuroma-like tissue.[3]

Ganglioneuroblastoma, Intermixed (Schwannian Stroma-rich) Category[#]
Ganglioneuromatous (stroma-rich) component of tumor exceeds 50%; intermixed or randomly distributed pattern of microscopic neuroblastic nests, consisting of cells in various stages of differentiation (neuroblasts, differentiating neuroblasts, maturing ganglion cells); abundant neuropil; macroscopic hemorrhagic nodules are absent.

Ganglioneuroma (Schwannian Stroma-dominant) Category
Two subtypes are included: neuroblastic cells (differentiating neuroblasts, maturing and mature ganglion cells) in the tumor tissue do not form microscopic nests but are individually distributed in the Schwannian stroma.

Maturing Subtype
Predominately ganglioneuromatous stroma; minor, scattered groups of differentiating neuroblasts or maturing ganglion cells along with completely mature ganglion cells.

Mature Subtype
Mature Schwannian stroma and ganglion cells; neuritic fascicular processes, accompanied by Schwann cells and perineurial cells; absence of neuroblastomatous component in complete maturation; satellite cells accompany mature ganglion cells.

Neuroblastoma (Schwannian Stroma-poor), Not Otherwise Specified (NOS)
Tumor diagnosis of neuroblastoma (Schwannian stroma-poor); subtyping not possible due to poor quality of sample or section.

Ganglioneuroblastoma, NOS
Tumor diagnosis of ganglioneuroblastoma (Schwannian stroma-rich); subtyping not possible due to a limited amount of tissue for evaluation or extensive calcification of tumor.

Neuroblastic Tumor, Unclassifiable
Neuroblastic cells evident; sample insufficient for categorization into one of the four basic types. A small biopsy taken from a large tumor can result in this designation.

[#] Ganglioneuroblastomas are highly variable in both number of neuroblasts and their extent of differentiation. Variability is seen between tumors, between microscopic fields in the same tumor, and occasionally between the primary and metastatic tumor. Ganglioneuroblastoma diagnostic criteria include (a) mature Schwannian stromal component with individually scattered mature and/or maturing ganglion cells and (b) a neuroblastic component.

D. Degree of Differentiation
Neuroblastomas (Schwannian stroma-poor) and the neuroblastic component of nodular-type ganglioneuroblastomas are further classified into 1 of 3 subtypes[1]:

Undifferentiated Subtype
Neuropil absent; no tumor cell differentiation; diagnosis relies heavily on ancillary techniques, such as immunohistochemistry, electron microscopy, and/or molecular/cytogenetic analysis.

Poorly Differentiated Subtype
Neuropil evident in background; less than 5% of tumor cells show features of differentiating neuroblasts (ganglion cell-like) with synchronous differentiation of the nucleus (enlarged, vesicular with a single prominent nucleolus) and the cytoplasm (conspicuous, eosinophilic or amphophilic, and twice the diameter of the nucleus).

Differentiating Subtype
Greater than 5% of tumor cells show evidence of differentiation (may be accompanied by mature ganglion-like cells), and neuropil is usually abundant; some tumors can show substantial Schwannian stromal formation, frequently at their periphery, and a transition zone between neuroblastomatous and ganglioneuromatous regions can develop (although this zone lacks well-defined borders and comprises less than 50% of the tumor).

E. Mitotic-Karyorrhectic Index
The mitotic-karyorrhectic index (MKI)[1,4] is the number of mitotic and karyorrhectic nuclei per 5000 neuroblastic cells. It is a useful prognostic indicator for tumors in the neuroblastoma (Schwannian stroma-poor) category and should be determined as an average of all tumor sections available. The method described by Joshi et al[5] can be used to calculate MKI without the need to count 5000 cells. In summary, cellular density is usually estimated under low power, and the tumor is classified as either a dense (700 to 900 cells per 400X high-power fields [HPF])[#], moderate (400 to 600 tumor cells per HPF)[#], sparse (100 to 300 cells per HPF)[#], or mixed category (a mixed tumor has variable cellularity under different HPF). Once categorized, random HPF are chosen to count mitotic and karyorrhectic cells. High-power fields on specimens in the mixed category are selected to be proportional to the cellular density in the specimen; for example, in a sample with 70% dense cellularity and 30% sparse cellularity, 70% of the HPF should be in dense areas and 30% in sparse areas. In highly cellular tumors, the MKI can be determined in 6 to 8 HPF, whereas in tumors with low cellularity and prominent neuropil, 20 or more HPF may be necessary. Specimens are assigned to 1 of 3 prognostic categories:

(1) Low MKI	Less than 100 mitotic and karyorrhectic cells/5000 tumor cells, or less than 2% of tumor consisting of mitotic and karyorrhectic cells
(2) Intermediate MKI	100 to 200 mitotic and karyorrhectic cells/5000 tumor cells, or 2% to 4% of tumor consisting of mitotic and karyorrhectic cells
(3) High MKI	Greater than 200 mitotic and karyorrhectic cells/5000 tumor cells, or more than 4% of tumor consisting of mitotic and karyorrhectic cells

[#]Numbers of neuroblastic cells in each HPF (denominator for MKI determination) can vary, based on the type of microscope used (some practice required for assessing the number of neuroblastic cells per HPF on a given microscope). Numbers listed above in the parentheses are for a standard microscope setup with regular oculars. With a super-wide-field type of ocular, there may be an increased number of cells (1200 to 1500 cells per HPF in a dense category).

F. Staging

The International Neuroblastoma Staging System (INSS) is accepted as universally applicable and should always be recorded for new patients.[5] The core of clinical staging is the size of the primary tumor, locoregional lymph node status, and the presence or absence of distant metastases.

International Neuroblastoma Staging System (INSS)

Stage 1	Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumor microscopically (nodes attached to and removed with the primary tumor may be positive).
Stage 2A	Localized tumor with incomplete gross excision; representative ipsilateral nonadherent lymph nodes negative for tumor microscopically.
Stage 2B	Localized tumor with or without complete gross excision, with ipsilateral nonadherent lymph nodes positive for tumor. Enlarged contralateral lymph nodes must be negative microscopically.
Stage 3	Unresectable unilateral tumor infiltrating across the midline, with or without regional lymph node involvement; or localized unilateral tumor with contralateral regional lymph node involvement; or midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement. The midline is defined as the vertebral column. Tumors originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.
Stage 4	Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs (except as defined for stage 4S).
Stage 4S	Localized primary tumor (as defined for stage 1, 2A, or 2B), with dissemination limited to skin, liver, and/or bone marrow (limited to infants less than 1 year of age). Marrow involvement should be minimal (ie, less than 10% of total nucleated cells identified as malignant by bone biopsy or by bone marrow aspirate). More extensive bone marrow involvement would be considered to be stage 4 disease. A MIBG scan (if performed) should be negative for disease in the bone marrow.

G. Prognostic Groups

Risk group assessment can be defined by clinical and biological variables. A simplified approach is described using either pathologic variables combined with age (Table 1)[2] or a compendium of biologic and clinical risk factors (Table 2).[6] Also included is a risk-grouping scheme for clinical trials of the Children's Oncology Group Neuroblastoma Studies (Table 3) based on the combination of clinical stage, age at diagnosis, *MYCN* status, histopathology classification, and DNA index. According to this scheme, patients are classified into the Low-, Intermediate-, or High-Risk group. As for the patients in the Intermediate-Risk group, protocol assignment for treatment of the individual cases is determined by further subclassification based on the combination of the above-mentioned risk factors and presence or absence of 1p deletion and/or 11q loss of heterozygosity (LOH).

The International Neuroblastoma Pathology Classification (INPC)[2] uses age, neuroblastic maturation, Schwannian stromal content, and MKI as prognostic indicators. Unfavorable indicators include undifferentiated neuroblastoma (especially in older

patients) and high MKI. An important revision was added in 2003.[3] The original INPC classified all tumors in the category of ganglioneuroblastoma, nodular as unfavorable.[2] The revised INPC distinguishes 2 prognostic subsets in this category, favorable and unfavorable, by applying the same age-linked histopathology evaluation to the nodular (neuroblastoma) components.[3]

Table 1. International Neuroblastoma Pathology Prognostic Classification (INPC)

Age	Favorable Histology Group	Unfavorable Histology Group
Any	Ganglioneuroma (Schwannian stroma-dominant) • maturing • mature	
	Ganglioneuroblastoma, intermixed (Schwannian stroma-rich)	
		Neuroblastoma (Schwannian stroma-poor) • undifferentiated and any MKI
<1.5 y	Neuroblastoma (Schwannian stroma-poor) • poorly differentiated and low or intermediate MKI • differentiating and low or intermediate MKI	Neuroblastoma (Schwannian stroma-poor) • poorly differentiated and high MKI • differentiating and high MKI
1.5 y to <5 y	Neuroblastoma (Schwannian stroma-poor) • differentiating and low MKI	Neuroblastoma (Schwannian stroma-poor) • poorly differentiated and any MKI • differentiating and intermediate or high MKI
≥5 y		Neuroblastoma (Schwannian stroma-poor) • any subtype and any MKI
	Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor), Favorable Subset[#]	Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor), Unfavorable Subset[#]

MKI indicates mitosis-karyorrhexis index.

[#] The neuroblastic nodule(s) of the ganglioblastoma, nodular subtype are graded with the INPC age-linked histopathology evaluation and based on that evaluation classified as favorable or unfavorable. For multinodular tumors, each nodule is graded separately and the least favorable nodule determines the classification.

Table 2. Biologic and Clinical Risk Factors and Groups in Neuroblastoma

Parameter	Low Risk	Intermediate Risk	High Risk
MYCN status	Normal	Normal	Amplified (>10 copies)
Ploidy	Hyperdiploid	Near-diploid	Near-diploid
	Near-triploid	Near-tetraploid	Near-tetraploid
17q gain	Rare	Common	Common
11q, 14q loss of heterozygosity (LOH)	Rare	Common	Rare
1p LOH	Rare	Uncommon	Common
TRK A expression	High	Low or absent	Low or absent
TRK B expression	Truncated	Low or absent	Low or absent
TRK C expression	High	Low or absent	Low or absent
Age	Usually <1 y	Usually >1 y	Usually 1 to 5 y
Stage	1, 2, 4S	Usually 3 or 4	Usually 3 or 4
Expected survival rate#	Greater than 95% with surgery alone	~90% with various intensities of chemotherapy	~40%

[#] Based on the experience of Children's Oncology Group Neuroblastoma Studies/Protocols.

Table 3. Risk Grouping Scheme for the Children's Oncology Group Neuroblastoma Study

Study	Stage	Age	*MYCN*	Ploidy[#]	INPC[##]	Other
Low Risk	1	any	any	any	any	
Low Risk	2a/2b	any	not amp	any	any	resection ≥50%
Intermediate Risk	2a/2b	0 – 12 y	not amp	any	any	resection <50% or biopsy only
High Risk	2a/2b	any	amp	any	any	any degree of resection
Intermediate Risk	3	<547 d	not amp	any	any	
Intermediate Risk	3	≥547 d – 12 y	not amp	any	FH	
High Risk	3	any	amp	any	any	
High Risk	3	≥547 d	not amp	any	UH	
High Risk	4	<365 d	amp	any	any	
Intermediate Risk	4	<365 d	not amp	any	any	
High Risk	4	365-<547 d	amp	any	any	
High Risk	4	365-<547 d	any	DI=1	any	
High Risk	4	365-<547 d	any	any	UH	
Intermediate Risk	4	365-<547 d	not amp	DI>1	FH	
High Risk	4	≥547 d	any	any	any	
Low Risk	4s	<365 d	not amp	DI>1	FH	asymptomatic
Intermediate Risk	4s	<365 d	not amp	any	any	symptomatic
Intermediate Risk	4s	<365 d	not amp	DI=1	any	asymp or symp
Intermediate Risk	4s	<365 d	not amp	any	UH	asymp or symp
Intermediate Risk	4s	<365 d	missing	missing	missing	asymp or symp
High Risk	4s	<365 d	amp	any	any	asymp or symp

[#] Ploidy: DNA index (DI) greater than 1 (hyperdiploid) or equal to 1 (diploid); hypodiploid tumors (with DI less than 1) will be treated as a tumor with DI greater than 1.

[##] INPC (International Neuroblastoma Pathology Classification): FH = favorable histology, UH = unfavorable histology.

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H. Molecular Classification/Genetics

MYCN Amplification

The most prognostically relevant genetic alteration in neuroblastoma is *MYCN* amplification. *MYCN* gene amplification is associated with high-risk neuroblastic tumors and poor patient prognosis. *MYCN* is a proto-oncogene located on the short arm of chromosome 2, the amplification of which leads to inhibiting cellular differentiation and promoting cellular proliferation and apoptosis/karyorrhexis.[7] Not surprisingly, amplification is associated with undifferentiated and poorly differentiated neuroblastomas with a high mitotic-karyorrhectic index (MKI).[8,9]

MYCN overexpression usually occurs by gene amplification in one or both of the following ways: (1) gene duplication adjacent to the usual locus on 2p, forming homogeneously staining regions (HSRs) seen on chromosomal banding patterns, and (2) formation of double minutes, small, circular extrachromosomal fragments of DNA that harbor copies of the *MYCN* gene and are replicated during mitosis. These mechanisms can occur individually or simultaneously in a given tumor cell.

The *MYCN* status of a given neuroblastic tumor can be determined by FISH within a relatively short period of time after the surgery/biopsy using touch preparation slides or formalin-fixed, paraffin-embedded sections (Note A). A double-staining procedure is recommended in order to compare the number of chromosome 2 and *MYCN* signals in the same tumor nuclei. Additional *MYCN* signals associated with a similar increase in the number of chromosome 2 signals does not represent *MYCN* amplification. *MYCN* status is defined as “amplified” when *MYCN* signals exceed chromosome 2 signals by 3 times or more in the given tumor cell nuclei. The prognostic significance of tumors showing increased *MYCN* signals, but not more than 3 times of chromosome 2 signals (*MYCN* gain) is yet to be determined.

MYCN amplification is also correlated with advanced-stage tumors often having chromosome 1p deletions, especially del 1p36.3.[10] The deletion of 14q has also been shown to be unfavorable, as have loss of 11q and gain of 17q.[11]

DNA Index

Determination of DNA index by flow cytometry is also important; however, a minimum of 100 mg and preferably 1 g of fresh tumor is typically required for this purpose (Note A). A DNA index near diploid/tetraploid is unfavorable, while hyperdiploid (near triploid) tumors have a better prognosis. However, the prognostic effects of DNA index are reported to be limited to those patients diagnosed at younger than 1 year of age.[11]

Others

Additional genetic abnormalities have clinicopathologic significance in neuroblastic tumors. Higher expression of TrkA (high-affinity nerve growth factor receptor) portends a good prognosis; *MYCN*-amplified tumors usually have a lower expression of TrkA.[1] Finally, recent studies have demonstrated mutations in the anaplastic lymphoma kinase (ALK) gene in a subset of neuroblastic tumors, as well as in the germline of patients with a familial predisposition to this disease.[12-14]

I. Clinical Presentation

The clinical presentation of neuroblastoma may provide valuable information in assessing biologic risk. The abdomen is the most common primary site of neuroblastoma, with more than 76% of tumors arising either in the adrenal glands or, less commonly, in the paravertebral sympathetic chains.[5]

The posterior mediastinum is the second most common primary site, and respiratory symptoms predominate. Cervical neuroblastoma presents as a mass with or without Horner syndrome.[15] All neuroblastomas, regardless of biologic risk, can extend along radicular nerves, through spinal foramina, and into the epidural space, forming a dumbbell-shaped mass. Because the spinal cord extends to the level of the T12 to L1 vertebrae, tumors above this level are more likely to cause cord compression and paralysis, bladder and bowel dysfunction, or numbness. Similarly, neuroblastomas primary in the pelvis may present with constipation or urinary symptoms, including dysuria, infection, flank pain, or urinary retention.[16]

The opsoclonus-myoclonus syndrome is the best example of a paraneoplastic manifestation of neuroblastoma. This is thought to occur due to cross-reactivity between antineuroblastoma antibodies and the Purkinje cells of the cerebellum. Although patients with opsoclonus-myoclonus syndrome usually have an excellent prognosis for their tumor, up to 70% of such patients will have permanent neurologic deficits despite complete tumor resection.[17]

J. Special Studies

Imaging

The most useful imaging study is computerized axial tomography (CT scan) performed with simultaneous administration of oral and intravenous contrast agents.[18] This provides excellent information about the primary tumor, including location, vascular encasement, and the status of regional lymph nodes. Hepatic and bony metastases can be visualized, as well as pulmonary metastases (the latter is an extremely rare site for dissemination).[5] Magnetic resonance imaging (MRI) can give valuable information about vascular and hepatic involvement and help to determine tumor resectability.

A diphosphate bone scan and a meta-iodobenzylguanide (MIBG) scan are requisite to assess the bone and bone marrow for distant disease.[19] Approximately 85% of neuroblastomas will take up MIBG.[5] A positive bone scan or bone survey indicates cortical bone involvement and is a negative prognostic factor.

Serum Chemistry

Serum chemistry assays are useful to help predict prognostic risk. These include serum lactic dehydrogenase (LDH), neuron-specific enolase (NSE), and ferritin.[20] Ferritin levels are the most important diagnostic marker of the three, with an elevation above normal (before transfusion) associated with a worse prognosis. Reference ranges are dependent on the individual laboratory, but an upper normal limit of 142 ng/mL frequently is reported.[21] Serial LDH levels correlate with disease activity, and pretreatment values of more than 1000 U/L are associated with a worse prognosis.[22] Serum levels of NSE more than 30 ng/mL also are associated with a worse prognosis.[23]

Endocrine Markers

Urinary catecholamine secretion is increased in neuroblastoma and is useful as a confirmatory diagnostic marker. Serial determinations are used to assess therapeutic response and identify recurrence. Vanillylmandelic acid (VMA) and homovanillic acid (HVA) are the two catecholamine metabolites commonly measured[24] via high-performance liquid chromatography. In one study,[25] the sensitivity and specificity of HVA for detection of neuroblastoma were 72% and 98%, respectively; corresponding figures for VMA were 80% sensitivity and 97% specificity. Urinary catecholamines may not be elevated in undifferentiated neuroblastomas.

References

1. Shimada H, Ambros IM, Dehner LP, Hata J, Joshi VV, Roald B. Terminology and morphologic criteria of neuroblastic tumors: recommendations by the International Neuroblastoma Pathology Committee. *Cancer.* 1999;86(2):349-363.
2. Shimada, H, Ambros IM, Dehner LP, et al. The International Neuroblastoma Pathology Classification (the Shimada system). *Cancer.* 1999;86(2):364-372.
3. Peuchmaur M, d'Amore ES, Joshi VV, et al. Revision of the International Neuroblastoma Pathology Classification: confirmation of favorable and unfavorable prognostic subsets in ganglioneuroblastoma, nodular. *Cancer.* 2003;98(10):2274-2281.
4. Joshi VV, Chatten J, Sather HN, Shimada H. Evaluation of the Shimada classification in advanced neuroblastoma with a special reference to the mitosis-karyorrhexis index: a report from the Children's Cancer Study Group. *Mod Pathol.* 1991;4(2):139-147.
5. LaQuaglia MP. Surgical management of neuroblastoma. *Semin Pediatr Surg.* 2001;10:132-139.
6. Joshi VV, Cantor AB, Altshuler G, et al. Age-linked prognostic categorization based on a new histologic grading system of neuroblastomas: a clinical pathologic study of 211 cases from the Pediatric Oncology Group. *Cancer.* 1992;69(8):2197-2211.
7. Wenzel A, Cziepluch C, Hamann U, Schurmann J, Schwab M. The N-Myc oncoprotein is associated in vivo with the phosphoprotein Max (p20/22) in human neuroblastoma cells. *EMBO J.* 1991;10(12):3703-3712.
8. Shimada H, Stram D, Chatten J, et al. Identification of subsets of neuroblastomas combined with histopathologic and N-myc analysis. *J Natl Cancer Inst.* 1995;87(19):1470-1476.
9. Goto S, Umehara S, Gerbing RB, et al. Histopathology and MYCN status in peripheral neuroblastic tumors: a report from the Children's Cancer Group. *Cancer.* 2001;92(10):2699-2708.
10. Attiyeh EF, London WB, Mossé YP, et al; Children's Oncology Group. Chromosome 1p and 11q deletions and outcome in neuroblastoma. *N Engl J Med.* 2005;353(21):2243-2253.
11. Look AT, Hayes FA, Shuster JJ, et al. Clinical relevance of tumor cell ploidy and N-myc gene amplification in childhood neuroblastoma: a Pediatric Oncology Group study. *J Clin Oncol.* 1991;9(4):581-591.
12. Mosse YP, Laudenslager M, Longo L, et al. Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature.* 2008;455(7215):930-936.

13. Janoueix-Lerosey I, Lequin D, Brugières L, et al. Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. *Nature*. 2008;455(7215):967-970.
14. Chen Y, Takita J, Choi YL, et al. Oncogenic mutations of ALK kinase in neuroblastoma. *Nature*. 2008;455(7215):971-974.
15. Abramson SJ, Berdon WE, Ruzal-Shapiro C, Stolar C, Garvin J. Cervical neuroblastoma in eleven infants—a tumor with favorable prognosis: clinical and radiologic (US, ST, MRI) findings. *Pediatr Radiol.* 1993;23(4):253-257.
16. Haase GM, O'Leary MC, Stram DO, et al. Pelvic neuroblastoma—implications for a new favorable subgroup: a Children's Cancer Group experience. *Ann Surg Oncol.* 1995;2(6):516-523.
17. Russo C, Cohn SL, Petruzzi MJ, et al. Long-term neurologic outcome in children with opsoclonus-myoclonus associated with neuroblastoma: a report from the Pediatric Oncology Group. *Med Pediatr Oncol.* 1997;28(4):284-288.
18. Hugosson C, Nyman R, Jorulf H, et al. Imaging of abdominal neuroblastoma in children. *Acta Radiol.* 1999;40(5):534-542.
19. Jacobs A, Delree M, Desprechins B, et al. Consolidating the role of *I-MIBG scintigraphy in childhood neuroblastoma: five years of clinical experience. *Pediatr Radiol.* 1990;20(3):157-159.
20. Hann HWL. Serum markers and prognosis in neuroblastoma: ferritin, LDH, and NSE. In: Brodeur GM, Sawada T, Tsuchida Y, Voute PA, eds. *Neuroblastoma*. Amsterdam, The Netherlands: Elsevier; 2000: 371-381.
21. Hann HW, Evans AE, Siegel SE, et al. Prognostic importance of serum ferritin in patients with stages III and IV neuroblastoma: the Children's Cancer Group experience. *Cancer Res.* 1985;45(6):2843-2848.
22. Berthold F, Trechow R, Utsch S, et al. Prognostic factors in metastatic neuroblastoma: a multivariate analysis of 182 cases. *Am J Pediatr Hematol Oncol.* 1992;14(3):207-215.
23. Zeltzer PM, Marangos PJ, Sather H, et al. Prognostic importance of serum neuropeptide specific enolase in local and widespread neuroblastoma. *Prog Clin Biol Res.* 1985;175:319-329.
24. Laug WE, Siegel SE, Shaw KN, Landing B, Baptista J, Gutenstein M. Initial urinary catecholamine metabolite concentrations and prognosis in neuroblastoma. *Pediatrics.* 1978;62(1):77-83.
25. Horsmans Y, Desager JP, Harvengt C. Sensitivity and specificity of the determination of urinary catecholamines and their acid metabolites in the diagnosis of neuroblastoma in children. *Bull Cancer.* 1990;77(10):985-989.