Neuroblastoma

Protocol applies to the examination of specimens from patients with neuroblastoma and related neuroblastic tumors.

Protocol date: July 2005
No AJCC/UICC staging system

Procedures
• Cytology (No Accompanying Checklist)
• Incisional Biopsy (Needle or Wedge) (No Accompanying Checklist)
• Resection

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Summary

This is a new protocol for 2005.

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 work-up of neuroblastoma and require at least 100 mg of viable snap-frozen tissue as the second priority for work-up (Note A).

For more information contact: The Children’s Oncology Group Biopathology Center, Phone: (614) 722-2890 or (800) 347-2486.
Surgical Pathology Cancer Case Summary (Checklist)

Protocol date: July 2005
Applies to neuroblastoma only
No AJCC/UICC staging system

NEUROBLASTOMA: Resection

Patient name:
Surgical pathology number:

* Note: Check 1 response unless otherwise indicated.

MACROSCOPIC

Specimen Type
___ Subtotal adrenalectomy
___ Total adrenalectomy
___ Other (specify): _________________________
___ Not specified

Tumor Site
Specify: _________________________
___ Not specified

Laterality (check all that apply)
___ Right
___ Left
___ Midline
___ Not specified

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

*Specimen Weight
*Specify: ___ g

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

Tumor Weight (if separate from total specimen)
Specify: ___ g

* Data elements with asterisks are not required for accreditation purposes for the Commission on Cancer. These elements may be clinically important, but are not yet validated or regularly used in patient management. Alternatively, the necessary data may not be available to the pathologist at the time of pathologic assessment of this specimen.
**MICROSCOPIC**

**Extent of Invasion**

**Primary Tumor**
___ Cannot be assessed
___ Encapsulated
___ Capsular extension without other organ involvement
___ Extension into other organs

**Regional Lymph Nodes** (check all that apply)
___ Cannot be assessed
___ No regional lymph node metastasis
___ Right regional lymph node metastasis
   Specify: Number examined: ___
   Number involved: ___
___ Left regional lymph node metastasis
   Specify: Number examined: ___
   Number involved: ___

**Distant Metastasis**
___ Cannot be assessed
___ Distant metastasis
   *Specify site(s): ___________________________

**Margins**
___ Cannot be assessed
___ Margins uninvolved by tumor
___ Margin(s) involved by tumor

**Venous/Lymphatic (Large/Small Vessel) Invasion**
*___ Absent
*___ Present
*___ Indeterminate

**International Neuroblastoma Pathology Classification**
___ Cannot be determined

**Favorable Histopathology**
___ Any age; ganglioneuroma (Schwannian stroma-dominant); maturing or mature
___ Any age; ganglioneuroblastoma, intermixed (Schwannian stroma-rich)
___ Less than 1.5 years old; neuroblastoma (Schwannian stroma-poor); poorly
differentiated and low or intermediate mitosis-karyorrhexis index (MKI)
___ 1.5 years up to less than 5 years old; neuroblastoma (Schwannian stroma-poor);
differentiating and low MKI

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Unfavorable Histopathology
___ Any age; ganglioneuroblastoma, nodular (Composite, Schwannian stroma-rich/stroma-dominant and stroma-poor)
___ Any age; neuroblastoma (Schwannian stroma-poor); undifferentiated and any MKI
___ Less than 1.5 years old; neuroblastoma (Schwannian stroma-poor); poorly differentiated and high MKI, or differentiating and high MKI
___ 1.5 years up to less than 5 years old; neuroblastoma (Schwannian stroma-poor); poorly differentiated and any MKI, or differentiating and intermediate or high MKI
___ Equal to or greater than 5 years old; neuroblastoma (Schwannian stroma-poor); any subtype and any MKI

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
• 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 of age)

# 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, 3M).

## The midline is defined as the vertebral column. Tumors originating on 1 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 MIBG scan (if performed) should be negative in the marrow.

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*Additional Pathologic Findings (check all that apply)
* ___ None identified
* ___ Tumor necrosis
* ___ Tumor calcification
* ___ Other (specify): ______________________

*Comment(s)
Background Documentation

Protocol date: July 2005

I. Cytologic Material
A. Clinical Information
1. Patient identification
   a. Name
   b. Identification number
   c. Age (birth date)
   d. Sex
2. Responsible physician(s)
3. Date of procedure
4. Other clinical information
   a. Relevant history (eg, previous diagnoses, treatment, family history) (Note B)
   b. Relevant findings (eg, imaging studies, including meta-iodobenzylguanidine [MIBG] scan; urinary catecholamines) (Notes C and D)
   c. Clinical diagnosis
   d. Procedure (eg, fine-needle aspiration [FNA])
   e. Anatomic sites(s) of specimen (eg, right/left adrenal gland, related sites)
B. Macroscopic Examination
1. Specimen
   a. Unfixed/fixed (specify fixative)
   b. Number of slides received
   c. Quantity and appearance of fluid specimen
   d. Other materials received (eg, touch preparation from tissue)
   e. Results of intraprocedural consultation
2. Material submitted for microscopic examination (eg, smear, cytocentrifuge, touch or filter preparation, cell block)
3. Special studies (specify) (eg, immunohistochemistry, molecular analysis, cytogenetic analysis) (Notes A and E)
C. Microscopic Evaluation
1. Adequacy of specimen (if unsatisfactory for evaluation, specify reason)
2. Tumor, if present
   a. Histologic category and subtype, if possible (Note F)
   b. Other features (eg, nuclear changes consistent with neuroblastic or ganglionic differentiation)
3. Other pathologic findings, if present (eg, necrosis, calcification)
4. Results/status of special studies (specify)
5. Comments
   a. Correlation with intraprocedural consultation, as appropriate
   b. Correlation with other specimens, as appropriate
   c. Correlation with clinical information, as appropriate
II. Incisional Biopsy  
(Any Surgical Approach Less Than Complete Adrenalectomy; or  
Other Primary Tumor Excision, Including Needle or Wedge Biopsy)

A. Clinical Information  
1. Patient identification  
   a. Name  
   b. Identification number  
   c. Age (birth date)  
   d. Sex  
2. Responsible physician(s)  
3. Date of procedure  
4. Other clinical information  
   a. Relevant history (eg, previous diagnoses, treatment, family history)  
   b. Relevant findings (eg, imaging studies, including MIBG scan; urinary catecholamines)  
   c. Clinical diagnosis  
   d. Procedure (eg, core needle biopsy, wedge biopsy)  
   e. Anatomic sites(s) of specimen (eg, right/left adrenal gland, related sites)

B. Macroscopic Examination (Note G)  
1. Specimen  
   a. Unfixed/fixed (specify fixative)  
   b. Number of pieces  
   c. Dimensions  
   d. Descriptive features (eg, hemorrhage, necrosis)  
   e. Orientation, if designated by surgeon  
   f. Results of intraoperative consultation  
2. Tissue submitted for microscopic examination, as appropriate  
   a. Entire specimen  
   b. Selected sample  
   c. Frozen section tissue fragment(s), unless saved for special studies  
3. Special studies (specify) (eg, immunohistochemistry, ploidy analysis, 
   fluorescense in situ hybridization [FISH], cytogenetic analysis) (Notes A and E)

C. Microscopic Evaluation (Note F)  
1. Tumor  
   a. Histologic category (Note F)  
   b. Histologic subtype (Note F)  
   c. Other features (eg, calcification, amount of neuropil)  
   d. Venous/lymphatic vessel invasion, if possible to determine  
   e. Mitosis-karyorrhexis index (MKI) of neuroblastoma category (Notes F and H)  
2. Additional pathologic findings, if present (eg, necrosis)  
3. Results/status of special studies (specify)  
4. Comments  
   a. Correlation with intraoperative consultation, as appropriate  
   b. Correlation with other specimens, as appropriate  
   c. Correlation with clinical information, as appropriate  
   d. Determination of prognostic group (favorable histology group versus  
      unfavorable histology group) according to the International Neuroblastoma  
      Pathology Classification (Note F)
III. Resection  
(Adrenalectomy or Other Primary Tumor Excision)  

A. Clinical Information  
1. Patient identification  
   a. Name  
   b. Identification number  
   c. Age (birth date)  
   d. Sex  
2. Responsible physician(s)/clinic(s)  
3. Date of procedure  
4. Other clinical information  
   a. Relevant history (Note B)  
      (1) previous diagnoses  
      (2) surgery and date(s)  
      (3) radiation and date(s)  
      (4) chemotherapy and date(s)  
      (5) others  
   b. Relevant findings (eg, imaging studies, including MIBG scan; urinary catecholamines) (Notes C and D)  
   c. Clinical diagnosis  
   d. Procedure (specify anatomic site[s]) (Note G)  
      (1) excision  
      (2) anatomical structures removed (eg, associated kidney)  
      (3) lymph node dissection  
   e. Operative findings (documentation of areas of concern marked by surgeon)  

B. Macroscopic Examination (Note G)  
1. Specimen  
   a. Organ/tissues included  
   b. Unfixed/fixed (specify fixative)  
   c. Size (3 dimensions)  
   d. Weight  
   e. Orientation, if indicated by surgeon  
   f. Descriptive features (eg, hemorrhage, necrosis)  
   g. Results of intraoperative consultation  
2. Tumor(s)  
   a. Anatomical site(s) involved by tumor  
   b. Size (3 dimensions)  
   c. Descriptive characteristics (eg, firm/soft, color, consistency, hemorrhage, necrosis, biopsy scars)  
   d. Anatomic extent (structures involved by tumor and depth of invasion)  
   e. Relation to margins  
   f. Additional tumors  
3. Additional pathologic findings, if present  
4. Lymph nodes, if submitted  
   a. Number  
   b. Location, if designated by surgeon  
5. Margins  
6. Stage (Note I)
7. Tissues submitted for microscopic examination
   a. Tumor (adequate sampling of all areas; 1 section for each centimeter of maximal tumor diameter and/or different gross appearances)
   b. Nodules
   c. Margins of resection
   d. All lymph nodes
   e. Other lesions
   f. Frozen section tissue fragment(s), unless saved for special studies
   g. Other organs/tissues

8. Special studies (specify) (eg, immunohistochemistry, ploidy analysis, FISH, cytogenetic analysis) (Notes A and E)

C. Microscopic Examination (Note F)
   1. Tumor
      a. Histologic category (Note F)
      b. Histologic subtype (Note F)
      c. Other descriptive features (eg, calcification, amount of neuropil)
      d. Venous/lymphatic vessel invasion, if possible to determine
      e. Mitosis-karyorrhexis index (MKI) of neuroblastoma category (Notes F and H)
      f. Evaluation of post-therapy tumors for differentiation, necrosis, and fibrosis
      g. Closest distance to margin
   2. Lymph nodes
      a. Number (location, if possible)
      b. Number involved by tumor
   3. Additional pathologic findings, if present
   4. Results/status of special studies (specify) (Notes A and E)
   5. Other organs/tissues
   6. Comments
      a. Correlation with intraoperative consultation, as appropriate
      b. Correlation with other specimens, as appropriate
      c. Correlation with clinical information, as appropriate
      d. Determination of prognostic group (favorable histology group versus unfavorable histology group) according to the International Neuroblastoma Pathology Classification (Note F)

Explanatory Notes

A. Molecular and Cytogenetic Testing
   MYCN gene amplification (greater than 10 copies by Southern blot or fluorescence in situ hybridization [FISH]) in neuroblastoma tumor links to a poor prognosis of the patient. The MYCN gene is located in the short arm of chromosome 2. When amplified, it forms double minutes (DMs) and homogeneously staining regions (HSRs), and produces excess amount of N-myc protein. The myc-max protein complex in the tumor cell nucleus has been shown to inhibit cellular differentiation and promote cellular proliferation and apoptosis/karyorrhexis. Hence, amplification is usually seen in undifferentiated and poorly differentiated neuroblastomas (Schwannian stroma-poor) and correlates with a higher mitosis-karyorrhexis index (MKI) (Note H). MYCN status of the tumor can be determined by the FISH method within a relatively short period of time after the surgery/biopsy. A double-staining procedure is recommended for comparing the number of chromosome 2 and MYCN signals in the same tumor nuclei for determining MYCN status. Increased MYCN signals associated with a like increase in
number of chromosome 2 signals does not represent MYCN amplification. MYCN signals must be seen in excess of chromosome 2 signals by an average of 10 signals per nucleus to be considered true MYCN amplification.

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

Determination of DNA index by flow cytometry also is very important. A DNA index near diploid/tetraploid is unfavorable, while hyperdiploid (near-triploid) tumors have a good prognosis. However, prognostic effects of DNA index are reported to be limited to those patients diagnosed at less than 1 year of age.5

Higher expression of TrkA (high-affinity nerve growth factor receptor) favors a good prognosis.1 MYCN-amplified tumors usually have a lower expression of TrkA.

While MYCN FISH studies can be performed on touch preparations, and ploidy analysis can be performed on frozen tissue also available for touch preparations, cytogenetics requires fresh tissue. A minimum of 100 mg and preferably 1 g of fresh tumor is required for these purposes (Note G).

B. 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 adrenals or, less commonly, in the paramidline sympathetic chains.1 In older children, an abdominal mass usually represents an adrenal primary tumor, whereas in infants, it often represents hepatomegaly secondary to metastatic disease.

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.6 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.7

The opsoclonus-myoclonus syndrome is a prime example of a paraneoplastic manifestation of neuroblastoma. Patients with this syndrome usually have an excellent prognosis. This is thought to be secondary to cross-reaction of antineuroblastoma antibodies with the Purkinje cells of the cerebellum. As many as 70% of such patients have permanent neurologic deficits despite curative tumor resection.8

C. Imaging Studies
Ultrasound scans are the most common initial screening study to confirm a palpable abdominal or pelvic mass.9 The most useful imaging study is computerized axial tomography (CT scan) done with simultaneous oral and intravenous contrast.10
gives excellent information about the primary tumor, including location, vascular encasement, and the status of regional lymph nodes. Hepatic and even gross bony metastases can be visualized, as can pulmonary metastases (the latter is an extremely rare site for dissemination). Magnetic resonance images (MRI) can give valuable information about vascular and hepatic involvement and help to determine tumor resectability but are difficult to perform in active young children.

A diphosphate bone scan and a meta-iodobenzylguanidine (MIBG) scan are requisite to assess the bone and bone marrow for distant disease. A positive bone scan or bone survey indicates cortical bone involvement and is a negative prognostic factor; these patients are at high risk. Approximately 85% of neuroblastomas will take up MIBG.

**D. Endocrine Studies**

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 2 catecholamine metabolites commonly measured via high-performance liquid chromatography. In 1 study, the sensitivity and specificity of HVA for detection of neuroblastoma were 72% and 98%; corresponding figures for VMA were 80% sensitivity and 97% specificity. On rare occasions, increased urinary catecholamine secretion may not be seen with an undifferentiated neuroblastoma.

Rarely, the first diagnostic sign of neuroblastoma may be hypersecretion of vasoactive intestinal peptide with associated watery diarrhea.

**E. Special Studies**

**Serology**

Serum determinations are useful to help predict prognostic risk. These include serum lactic dehydrogenase (LDH), neuron-specific enolase (NSE), and ferritin. Ferritin levels are the most important diagnostic marker of the 3, 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. Serial LDH levels correlate with disease activity, and pretreatment values of more than 1000 U/L are associated with a worse prognosis. Serum levels of NSE more than 30 ng/mL also are associated with a worse prognosis.

**Immunohistochemistry**

Schwann cells: S-100 protein-positive.

The following are positive in a variable proportion of cases:

- Neuron-specific enolase
- Chromogranin A
- Synaptophysin
- Tyrosine hydroxylase
- Protein gene product 9.5
- GD2 (disialoganglioside, a ganglioside on human neuroblastoma cell membrane)
- NB84
The following are usually negative:
- Actin
- Desmin
- Low-molecular-weight cytokeratin
- CD45 (leukocyte common antigen)
- Vimentin

**Differential Diagnosis**
A cell surface glycoprotein, p30/32 (product of the MIC2 gene detected by CD99 antibodies), common in peripheral primitive neuroectodermal tumor (pPNET)/Ewing sarcoma, usually is negative in neuroblastoma. In contrast, tyrosine hydroxylase commonly is positive for neuroblastoma and negative for pPNET/Ewing sarcoma.

Undifferentiated cells (in poorly differentiated subtype) may, on rare occasions, express vimentin and have rhabdoid morphology.

**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.

**F. Morphologic Categories**
It is recommended that the International Neuroblastoma Classification described below be used when describing tumor samples.

There are 4 categories in this group of tumors:

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

Within each category, 1 or more subtypes are recognized.

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; whereas tumors in the ganglioneuroblastoma, intermixed category and the ganglioneuroma category are characterized by a presence of ganglioneuromatous tissue, where mature and/or maturing ganglion cells are individually scattered in a background of highly developed Schwannian stroma. Tumors in the ganglioneuroblastoma, nodular category are composed of multiple clones: one shows an appearance of either ganglioneuroblastoma, intermixed or ganglioneuroma, and the other(s) show that (those) of neuroblastoma.
**Neuroblastoma (Schwannian Stroma-poor) Category**

Three subtypes are distinguished.

**Undifferentiated Subtype**

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

**Poorly-differentiated Subtype**

Neuropil background evident; 5% or fewer tumor cells show a feature of differentiating neuroblasts with a synchronous differentiation of nucleus (enlarged, vesicular with a single prominent nucleolus) and cytoplasm (conspicuous, eosinophilic or amphophilic, and 2 times larger in diameter than nucleus).

**Differentiating Subtype**

*Greater than 5% of tumor cells show an appearance of differentiating neuroblasts (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 region can develop, although this zone does not have well-defined borders and comprises less than 50% of the tumor.*

**Ganglioneuroblastoma, Intermixed (Schwannian Stroma-rich) Category**

Ganglioneuromatous (stroma-rich) component of tumor exceeds 50%; intermixed or randomly distributed pattern of microscopic neuroblastic nests present, 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.

**Ganglioneuroblastoma, Nodular (Composite Schwannian Stroma-rich/ Stromadominant and Stroma-poor) Category**

Ganglioneuroblastoma, intermixed (stroma-rich) or ganglioneuroma (stroma-dominant) with macroscopic neuroblastic nodules (stroma-poor and usually hemorrhagic); border between nodule and stroma-rich or stroma-dominant region is often abrupt microscopically, but may instead be more gradual; the neuroblastoma component may be found in a metastatic tumor where the primary is ganglioneuroblastoma, intermixed or ganglioneuroma.
Neuroblastic Tumor, Unclassifiable
Neuroblastic cells evident; sample insufficient for categorization into 1 of the 4 basic types. A small biopsy taken from a large tumor can result in this designation.

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.

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.

The presence of calcification, in any amount in the tumor tissue, tends to indicate an improved prognosis of the patient.21

Differential Diagnosis
Primitive rhabdomyosarcoma
Peripheral primitive neuroectodermal tumor (pPNET)/Ewing sarcoma
Blastematous Wilms tumor
Blastic hematopoietic neoplasm
Malignant rhabdoid tumor
Desmoplastic small-round cell tumor

G. Sampling
In the complete macroscopic evaluation of the 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 the inked surgical margins).19

The genetic and morphologic heterogeneity of neuroblastoma requires extensive biologic and histologic study of the tumor. If practical, the entire tumor should be examined microscopically to facilitate the detection of any neuroblastic nodules that may have been overlooked on gross examination. In addition to the tissue taken for histologic examination as described, the International Neuroblastoma Pathology Committee recommends sampling a neuroblastic surgical specimen for biologic studies as follows.19
A minimum of 2 samples (A and B, each 1x1x1 cm) should be taken, preferably from morphologically different areas. Samples A and B are split into 4 pieces:

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1 2
3 4
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**A,B 1**  
Make at least 10 touch preparations (air-dried, unfixed, and, if necessary, stored at −20°C) for in situ hybridization (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.\(^{19}\)

**H. Mitosis-Karyorrhexis Index\(^{19,22}\)**  
The mitosis-karyorrhexis index (MKI) is the number of mitoses 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\(^{22}\) 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 field [HPF])\(^9\), moderate (400 to 600 tumor cells per HPF)\(^9\), sparse (100 to 300 cells per HPF)\(^9\), or mixed category (a mixed tumor has variable cellularity under different high-power fields). Once categorized, random HPFs are chosen to count mitotic and karyorrhectic cells. HPFs 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 HPFs should be in dense areas and 30% in sparse areas. In highly cellular tumors, the MKI can be determined in 6 to 8 HPFs, whereas in tumors with low cellularity and prominent neuropil, 20 or more HPFs may be necessary. Specimens are assigned to 1 of 3 prognostic categories:
Neuroblastoma • Pediatric

(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 greater 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 your microscope (some practice required for assessing the number of neuroblastic cells in your HPF). Numbers listed above in the parentheses are for the microscope with a regular ocular. With a super-wide-field type of ocular, you may be able to have 1200 to 1500 cells per HPF in a dense category.

I. Staging
The International Neuroblastoma Staging System (INSS) is accepted as universally applicable and should always be recorded for new patients.¹ The core of clinical staging is the size of the primary tumor, locoregional lymph node status, and the presence 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 1 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. The results of the MIBG scan (if performed) should be negative for disease in the bone marrow.
J. 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)\textsuperscript{20,23} or a compendium of biologic and clinical risk factors (Table 2).\textsuperscript{1} 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 (only for infants) (Table 3).

The International Neuroblastoma Pathology Classification\textsuperscript{20} uses age, neuroblastic maturation, and MKI as prognostic indicators. Unfavorable indicators include undifferentiated neuroblastoma (especially in older patients) and high MKI.
Table 1. International Neuroblastoma Pathology Prognostic Classification

<table>
<thead>
<tr>
<th>Age</th>
<th>Favorable Histology Group</th>
<th>Unfavorable Histology Group</th>
</tr>
</thead>
</table>
| Any                | Ganglioneuroma (Schwannian stroma-dominant) • maturing • mature | Ganglioneuroblastoma, nodular (Composite, Schwannian stroma-rich/stroma-dominant and stroma-poor)
|                    | Ganglioneuroblastoma, intermixed (Schwannian stroma-rich)      | Neuroblastoma (Schwannian stroma-poor) • undifferentiated and any MKI                     |
| Less than 1.5 years| Neuroblastoma (Schwannian stroma-poor) • poorly differentiated and low or intermediate MKI | Neuroblastoma (Schwannian stroma-poor) • poorly differentiated and high MKI • differentiating and high MKI |
| 1.5 years up to less than 5 years | Neuroblastoma (Schwannian stroma-poor) • differentiating and low MKI | Neuroblastoma (Schwannian stroma-poor) • poorly differentiated and any MKI • differentiating and intermediate or high MKI |
| Equal to or greater than 5 years | | Neuroblastoma (Schwannian stroma-poor) • any subtype and any MKI |

# All tumors in the category of ganglioneuroblastoma, nodular are classified into an unfavorable histology group according to the original Shimada classification and the International Neuroblastoma Pathology Classification. However, recent analysis distinguished 2 prognostic subsets, favorable and unfavorable, by applying the same age-linked histopathology evaluation (see Table 1) to the nodular (neuroblastoma) components of the tumors in this category. The International Neuroblastoma Pathology Committee has approved the presence of these 2 subsets and is currently preparing a new version of the Classification, with modification accordingly.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low Risk</th>
<th>Intermediate Risk</th>
<th>High Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYCN status</td>
<td>Normal</td>
<td>Normal</td>
<td>Amplified (greater than 10 copies)</td>
</tr>
<tr>
<td>Ploidy</td>
<td>Hyperdiploid</td>
<td>Near-diploid</td>
<td>Near-diploid</td>
</tr>
<tr>
<td></td>
<td>Near-triploid</td>
<td>Near-tetraploid</td>
<td>Near-tetraploid</td>
</tr>
<tr>
<td>17q gain</td>
<td>Rare</td>
<td>Common</td>
<td>Common</td>
</tr>
<tr>
<td>11q, 14q loss of heterozygosity (LOH)</td>
<td>Rare</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td>1p LOH</td>
<td>Rare</td>
<td>Uncommon</td>
<td>Common</td>
</tr>
<tr>
<td>TRK A expression</td>
<td>High</td>
<td>Low or absent</td>
<td>Low or absent</td>
</tr>
<tr>
<td>TRK B expression</td>
<td>Truncated</td>
<td>Low or absent</td>
<td>Low or absent</td>
</tr>
<tr>
<td>TRK C expression</td>
<td>High</td>
<td>Low or absent</td>
<td>Low or absent</td>
</tr>
<tr>
<td>Age</td>
<td>Usually less than 1 year</td>
<td>Usually greater than 1 year</td>
<td>Usually 1 to 5 years</td>
</tr>
<tr>
<td>Stage</td>
<td>1, 2, 4S</td>
<td>Usually 3 or 4</td>
<td>Usually 3 or 4</td>
</tr>
<tr>
<td>3-year survival rate</td>
<td>Greater than 90%</td>
<td>30% to 50%</td>
<td>Less than 20%</td>
</tr>
</tbody>
</table>
Table 3. Risk Grouping Scheme for the Children’s Oncology Group Neuroblastoma Study

<table>
<thead>
<tr>
<th>INSS Stage</th>
<th>Age</th>
<th>MYCN Status</th>
<th>Shimada Histology</th>
<th>DNA Ploidy</th>
<th>Risk Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-21y</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>Low</td>
</tr>
<tr>
<td>2A/2B</td>
<td>Less than 365d</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>Between 1y-21y</td>
<td>Nonamplified</td>
<td>Any</td>
<td>N/A</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>Between 1y-21y</td>
<td>Amplified</td>
<td>Favorable</td>
<td>N/A</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>Between 1y-21y</td>
<td>Amplified</td>
<td>Unfavorable</td>
<td>N/A</td>
<td>High</td>
</tr>
<tr>
<td>3</td>
<td>Less than 365d</td>
<td>Nonamplified</td>
<td>Any</td>
<td>Any</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>Less than 365d</td>
<td>Amplified</td>
<td>Any</td>
<td>Any</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>Between 1y-21y</td>
<td>Nonamplified</td>
<td>Favorable</td>
<td>N/A</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Between 1y-21y</td>
<td>Nonamplified</td>
<td>Unfavorable</td>
<td>N/A</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Between 1y-21y</td>
<td>Amplified</td>
<td>Any</td>
<td>N/A</td>
<td>High</td>
</tr>
<tr>
<td>4</td>
<td>Less than 365d</td>
<td>Nonamplified</td>
<td>Any</td>
<td>Any</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>Less than 365d</td>
<td>Amplified</td>
<td>Any</td>
<td>Any</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Between 1y-21y</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>High</td>
</tr>
<tr>
<td>4S</td>
<td>Less than 365d</td>
<td>Nonamplified</td>
<td>Favorable</td>
<td>DI&gt;1#</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>Less than 365d</td>
<td>Nonamplified</td>
<td>Any</td>
<td>DI=1#</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>Less than 365d</td>
<td>Nonamplified</td>
<td>Unfavorable</td>
<td>Any</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>Less than 365d</td>
<td>Amplified</td>
<td>Any</td>
<td>Any</td>
<td>High</td>
</tr>
</tbody>
</table>

# DNA ploidy: DNA index (DI) greater than 1 (aneuploid) or equal to 1 (diploid); hypodiploid tumors (with DI less than 1) will be treated as a tumor with DI greater than 1.
References


Bibliography