Protocol for the Examination of Specimens From Patients With Neuroblastoma and Related Neuroblastic Tumors

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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. 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 the document. 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.

PROTOCOL FOR THE EXAMINATION OF SPECIMENS FROM PATIENTS WITH NEUROBLASTOMA AND RELATED NEUROBLASTIC TUMORS

This protocol applies to neuroblastoma and related neuroblastic tumors only. There is no American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) TNM classification system for the staging of neuroblastoma. The International Neuroblastoma Pathology Classification and International Neuroblastoma Staging System are recommended.

Important Note.—First priority should always be given to formalin-fixed tissue for morphologic evaluation. Special studies (eg, ploidy analysis, fluorescence in situ hybridization [FISH]) 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; telephone: (614) 722-2890 or (800) 347-2486.

Background Documentation

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)
         e. Anatomic site(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, immunohisto-chemistry, 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 site(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, immunohisto-chemistry, ploidy analysis, 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 vs 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) anatomic 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. Anatomic 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 of neuroblastoma category (notes **F** and **H**)
      f. Evaluation of posttherapy 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 vs unfavorable histology group) according to the International Neuroblastoma Pathology Classification (note **F**)

EXPLANATORY NOTES

A: Molecular and Cytogenetic Testing.[1]—*MYCN* gene amplification (>10 copies by Southern blot or 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 and homogeneously staining regions and produces an 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.[2] Hence, amplification is usually seen in undifferentiated and poorly differentiated neuroblastomas (Schwannian stroma-poor) and correlates with a higher MKI[3,4] (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 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, whereas 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.

Although *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 done with simultaneous oral and intravenous contrast.[10] This 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).[1] Magnetic resonance imaging can give valuable information about vascular and hepatic involvement and can help to determine tumor resectability, but it is difficult to perform in active young children.

A diphosphate bone scan and a MIBG scan are requisite to assess the bone and bone marrow for distant disease.[11] 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.[1]

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 and homovanillic acid are the 2 catecholamine metabolites commonly measured[12] via high-performance liquid chromatography. In 1 study,[13] the sensitivity and specificity of homovanillic acid for detection of neuroblastoma were 72% and 98%, respectively; corresponding figures for vanillylmandelic acid 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.[14]

E: Special Studies.—*Serology.*—Serum determinations are useful to help predict prognostic risk. These include serum lactic dehydrogenase, neuron-specific enolase, and ferritin.[15] 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.[16] Serial lactic dehydrogenase levels correlate with disease activity, and pretreatment values of more than 1000 U/L are associated with a worse prognosis.[17] Serum levels of neuron-specific enolase more than 30 ng/mL also are associated with a worse prognosis.[18]

Immunohistochemistry.—Schwann cells: S100 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/Ewing sarcoma, usually is negative in neuroblastoma. In contrast, tyrosine hydroxylase commonly is positive for neuroblastoma and negative for peripheral primitive neuroectodermal tumor/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[19,20] described later should 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: 1 shows an appearance of either ganglioneuroblastoma, intermixed or ganglioneuroma, and the other(s) show appearance 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.—More 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/Stroma-Dominant 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.—Tumor diagnosis of neuroblastoma (Schwannian stroma-poor); subtyping not possible because of poor quality of sample or section.

Ganglioneuroblastoma, Not Otherwise Specified.—Tumor diagnosis of ganglioneuroblastoma (Schwannian stroma-rich); subtyping not possible because of 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 tumors. Ganglioneuroblastoma diagnostic criteria include (1) mature Schwannian stromal component with individually scattered mature and/or

maturing ganglion cells, and (2) 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/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 1 × 1 × 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 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 MKI is the

Table 1. International Neuroblastoma Pathology Prognostic Classification[20]*

Age	Favorable Histology Group	Unfavorable Histology Group
Any	Ganglioneuroma (Schwannian stroma-dominant) ● maturing ● mature Ganglioneuroblastoma, intermixed (Schwannian stroma-rich) 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† Neuroblastoma (Schwannian stroma-poor) ● undifferentiated and any MKI
<1.5 y	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 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

* MKI indicates mitosis-karyorrhexis index.

† All tumors in the category of ganglioneuroblastoma, nodular were classified into an unfavorable histology group according to the original Shimada classification[24] and the International Neuroblastoma Pathology Classification.[20] However, recent analysis distinguished 2 prognostic subsets, favorable and unfavorable, by applying the same age-linked histopathology evaluation to the nodular (neuroblastoma) components of the the tumors in this category.[25] The International Neuroblastoma Pathology Committee has approved the presence of these 2 subsets and published a new version of the Classification, with modification accordingly.[26]

Table 2. Biologic and Clinical Risk Factors and Groups in Neuroblastoma[1]

Parameter	Low Risk	Intermediate Risk	High Risk
MYCN status	Normal	Normal	Amplified (>10 copies)
Ploidy	Hyperdiploid Near-triploid	Near-diploid Near-tetraploid	Near-diploid Near-tetraploid
17q gain	Rare	Common	Common
11q, 14q 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 1–5 y
Stage	1, 2, 4S	Usually 3 or 4	Usually 3 or 4
3-y survival rate	>90%	30%–50%	<20%

* LOH indicates loss of heterozygosity.

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

INSS Stage	Age	MYCN Status	Shimada Histology	DNA Ploidy	Risk Group
1	0–21 y	Any	Any	Any	Low
2A/2B	<365 d	Any	Any	Any	Low
	1–21 y	Nonamplified	Any	N/A	Low
	1–21 y	Amplified	Favorable	N/A	Low
	1–21 y	Amplified	Unfavorable	N/A	High
3	<365 d	Nonamplified	Any	Any	Intermediate
	<365 d	Amplified	Any	Any	High
	1–21 y	Nonamplified	Favorable	N/A	Intermediate
	1–21 y	Nonamplified	Unfavorable	N/A	High
	1–21 y	Amplified	Any	N/A	High
4	<365 d	Nonamplified	Any	Any	Intermediate
	<365 d	Amplified	Any	Any	High
	1–21 y	Any	Any	Any	High
4S	<365 d	Nonamplified	Favorable	DI > 1†	Low
	<365 d	Nonamplified	Any	DI = 1†	Intermediate
	<365 d	Nonamplified	Unfavorable	Any	Intermediate
	<365 d	Amplified	Any	Any	High

* INSS indicates International Neuroblastoma Staging System; N/A, not applicable; and DI, DNA index.

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

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 (700–900 cells/×400 high-power field [HPF]),* moderate (400–600 tumor cells/HPF),* sparse (100–300 cells/HPF),* or mixed category (a mixed tumor has variable cellularity under different HPFs). Once categorized, random HPFs 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 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:

(1) Low MKI	Fewer 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	More 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 of tumor cells 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 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

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, <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 biologic variables. A simplified approach is described using either pathologic variables combined with age (Table 1)[20,23] or a compendium of biologic and clinical risk factors (Table 2).[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[20] uses age, neuroblastic maturation, and MKI as prognostic indicators. Unfavorable indicators include undifferentiated neuroblastoma (especially in older patients) and high MKI.

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SURGICAL PATHOLOGY CANCER CASE SUMMARY (CHECKLIST)

Neuroblastoma: Resection

Patient Name: _____

Surgical Pathology Number: _____

MACROSCOPIC (check 1 response unless otherwise indicated)

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: ___ × ___ cm

*Specimen Weight
*Specify: ___ g

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

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

MICROSCOPIC (check 1 response unless otherwise indicated

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)
___ Any age; ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor), favorable subset
___ Less than 1.5 years old; neuroblastoma (Schwannian stroma-poor); poorly differentiated and low or intermediate mitosis-karyorrhexis index (MKI)
___ 1.5 years to less than 5 years old; neuroblastoma (Schwannian stroma-poor); differentiating and low MKI

Unfavorable Histopathology
___ Any age; ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor), unfavorable subset
___ 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 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†
(check all that apply under the appropriate stage)**

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)

† 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).

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

‡ 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 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.

*Additional Pathologic Findings (check all that apply)

*___ None identified

*___ Tumor necrosis

*___ Tumor calcification

*___ Other (specify): _____

*Comment(s) _____

_____

_____

* 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.