Protocol for the Examination of Biopsy Specimens From Patients With Neuroblastoma

Version: 4.1.0.0
Protocol Posting Date: June 2021
The use of this protocol is recommended for clinical care purposes but is not required for accreditation purposes.

This protocol should be used for the following procedures AND tumor types:

Procedure	Description
Biopsy	Includes specimens designated needle biopsy, incisional biopsy, or other
Tumor Type	**Description**
Neuroblastoma	Includes pediatric patients with neuroblastoma and related neuroblastic tumors

The following should NOT be reported using this protocol:

Procedure
Resection (consider Neuroblastoma Resection protocol)

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With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.
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Accreditation Requirements
The use of this case summary is recommended for clinical care purposes but is not required for accreditation purposes. The core and conditional data elements are routinely reported. Non-core data elements are indicated with a plus sign (+) to allow for reporting information that may be of clinical value.

Summary of Changes

v 4.1.0.0

- General Reformatting
- Made repeating question for nodular subtypes
- Update Pathologic Stage Section
- Elements that are recommended for clinical care purposes are designated as Core and Conditional (indicated by bolded text), while Non-core elements are now indicated with a plus (+) sign

Reporting Template

Protocol Posting Date: June 2021
Select a single response unless otherwise indicated.

CASE SUMMARY: (NEUROBLASTOMA: Biopsy)
First priority should always be given to formalin-fixed tissue for morphologic evaluation. Special studies (e.g., ploidy analysis, fluorescence in situ hybridization for MYCN status) 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.

CLINICAL

Patient Age
___ Less than 18 months
___ Greater than or equal to 18 months and less than 5 years
___ Greater than or equal to 5 years

SPECIMEN

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

TUMOR

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

Histologic Type (Note C)
___ Neuroblastoma
 # 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.
___ Ganglioneuroblastoma, nodular subtype#
Number of Nodules
___ Specify number: _____
___ Other (specify): _____
___ Cannot be determined (explain): _____

Nodular Subtype (specify for each nodule): _____
___ Ganglioneuroblastoma, intermixed subtype
___ Ganglioneuroma
___ Ganglioneuroma, maturing

___ Ganglioneuroma, mature
___ Neuroblastic tumor, unclassifiable
___ Cannot be determined (explain): _____
+Histologic Type Comment: _____

Degree of Differentiation (neuroblastic component) (Note D)
___ Undifferentiated
___ Poorly differentiated
___ Differentiating
___ Cannot be determined (explain): _____
___ Not applicable: _____

Mitotic-Karyorrhectic Index (MKI) (Note E)
Report neuroblastic component if not previously treated.
___ Low (less than 100 per 5000 cells; less than 2%)
___ Intermediate (100-200 per 5000 cells; 2%-4%)
___ High (greater than 200 per 5000 cells; greater than 4%)
___ Cannot be determined (explain): _____
___ Not applicable: _____

+Tumor Comment: _____

PATHOLOGIC STAGE

International Neuroblastoma Pathology Classification (INPC) (Note F)
INPC applies to untreated primary tumors and tumors in metastatic sites provided that there is sufficient material to classify histologically. Classification based on limited material (biopsy or incomplete resection) may be subject to sampling error and should be noted, accordingly. Bone marrow biopsy is useful only for evaluation of degree of neuroblastic differentiation, but is not eligible for MKI determination.
___ Favorable histopathology
- ___ Neuroblastoma, poorly differentiated subtype, low or intermediate MKI, less than 18 months old
- ___ Neuroblastoma, differentiating subtype, intermediate MKI, less than 18 months old
- ___ Neuroblastoma, differentiating subtype, low MKI, less than 5 years old
- ___ Ganglioneuroblastoma, nodular, poorly differentiated subtype, low or intermediate MKI, less than 18 months old
- ___ Ganglioneuroblastoma, nodular, intermediate MKI, less than 18 months old
- ___ Ganglioneuroblastoma, nodular, differentiating subtype, low MKI, less than 5 years old
- ___ Ganglioneuroblastoma, intermixed, any age
- ___ Ganglioneuroma, mature or maturing, any age

___ Unfavorable histopathology
- ___ Neuroblastoma, undifferentiated subtype, any MKI, any age
- ___ Neuroblastoma, poorly differentiated subtype, high MKI, any age
- ___ Neuroblastoma, poorly differentiated subtype, low or intermediate MKI, greater than 18 months old
- ___ Neuroblastoma, differentiating subtype, high MKI, any age
- ___ Neuroblastoma, differentiating subtype, low MKI, greater than 5 years old
- ___ Ganglioneuroblastoma, nodular, undifferentiated subtype, any MKI, any age
- ___ Ganglioneuroblastoma, nodular, poorly differentiated subtype, high MKI, any age
- ___ Ganglioneuroblastoma, nodular, poorly differentiated subtype, low or intermediate MKI, greater than 18 months old

___ Ganglioneuroblastoma, nodular, differentiating subtype, intermediate MKI, greater than 18 months old
___ Ganglioneuroblastoma, nodular, differentiating subtype, high MKI, any age
___ Ganglioneuroblastoma, nodular, differentiating subtype, low MKI, greater than 5 years old
___ Cannot be determined secondary to insufficient material

ADDITIONAL FINDINGS

+Additional Findings (specify) (Notes G,H): _____

SPECIAL STUDIES (Note H)

MYCN Amplification Status (required for all tumors except ganglioneuroma) (Note I)
Results of MYCN amplification information may not be available to the pathologist at the time of the report.
___ Not applicable
___ Not amplified
___ Amplified: _____
___ Gain: _____
___ Cannot be determined: _____
___ Pending

+Other Ancillary Studies (specify): _____

COMMENTS

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, 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, as below:

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) (for 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]

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.

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). Histologic classification based on limited material should be noted in these cases. 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.

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.

C. Histopathologic Type

It is recommended that the International Neuroblastoma Classification[1,2] described below be used when describing untreated 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 less than 50% of the tumor).[1]

Differential Diagnosis

The differential diagnosis of neuroblastoma usually also includes the pediatric small round blue cell tumors: Ewing sarcoma (including peripheral primitive neuroectodermal tumor [pPNET]), 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 primitive neuroectodermal tumor Ewing sarcoma and lymphomas, usually is negative in neuroblastoma; both neuroblastoma and Ewing sarcoma are frequently positive for PGP9.5 and NB84. In contrast, tyrosine hydroxylase and PHOX2B are commonly positive in neuroblastoma and negative in 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. Neuroblasts are also typically positive for synaptophysin and neuron-specific enolase, although these are less specific. Schwann cells are positive for S100 protein.

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: 1 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 (Schwannian stroma-rich) component of the tumor exceeds 50%. Neuroblastic component is present in an intermixed or randomly distributed pattern of microscopic neuroblastic nests. The neuroblastic component consists of cells in various stages of differentiation (neuroblasts, differentiating neuroblasts, maturing ganglion cells) and has varying amounts of 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
Schwannian stroma is predominant with minor, scattered groups of differentiating neuroblasts or maturing ganglion cells along with completely mature ganglion cells. There are no islands of neuropil.

Mature Subtype
Schwannian stroma predominant with exclusively completely mature ganglion cells. May have neuritic fascicular processes accompanied by Schwann cells and perineurial cells. Satellite cells may accompany mature ganglion cells. There is a complete absence of a neuroblastomatous component, including no islands of neuropil.

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.

[#] 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.

Post-chemotherapy specimens
Neuroblastomas may undergo extensive morphologic changes post-chemotherapy. For this reason, resections of treated tumors should be simply referred to as neuroblastoma with treatment effect, with reference to the original diagnostic subtype, if known. Similarly, recurrent disease should not be re-classified.

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.

D. Degree of Differentiation

Degree of differentiation should be applied to the initial diagnostic material (eg. pre-chemotherapy). 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).

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.

E. Mitotic-Karyorrhectic Index

The mitotic-karyorrhectic index (MKI)[1,2] is the number of mitotic and karyorrhectic nuclei per 5000 neuroblastic cells. At initial diagnosis, 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[2] 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 [HPFs])[#], 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 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 HPF 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	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%-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 microscope used (some practice is required for assessing the number of neuroblastic cells per HPF on a given microscope). The range of cells per*

HPF listed in parentheses in the above discussion 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).

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. 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.

F. Prognostic Groups

The International Neuroblastoma Pathology Classification (INPC)[1] 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.[2] The original INPC classified all tumors in the category of ganglioneuroblastoma, nodular, as unfavorable.[1] 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[2] (Table 1).

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 mitotic-karyorrhectic index (MKI)
Less than 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 greater than 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
Greater than or equal to 5 y	Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor), favorable subset[#]	Neuroblastoma (Schwannian stroma-poor) • any subtype and any MKI Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor), unfavorable subset[#]

[#] *The neuroblastic nodule(s) of the ganglioneuroblastoma, 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.*

References
1. Shimada, H, Ambros IM, Dehner LP, et al. The International Neuroblastoma Pathology Classification (the Shimada system). Cancer. 1999;86(2):364-372.
2. 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.

G. 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.[1]

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. 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.

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.

References
1. LaQuaglia MP. Surgical management of neuroblastoma. Semin Pediatr Surg. 2001;10:132-139.

H. Special Studies

Imaging

The most useful imaging study is computerized axial tomography (CT scan) performed with simultaneous administration of oral and intravenous contrast agents. 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).[1] Magnetic resonance imaging (MRI) can give valuable information about vascular and hepatic involvement and can help to determine tumor resectability.

A diphosphate bone scan and an MIBG scan are requisite to assess the bone and bone marrow for distant disease.[2] Approximately 85% of neuroblastomas will take up MIBG.[1] A positive bone scan or bone survey indicates cortical bone involvement and is a negative prognostic factor.

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 2 catecholamine metabolites commonly measured[3] via high-performance liquid chromatography. In 1 study[4], 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. LaQuaglia MP. Surgical management of neuroblastoma. Semin Pediatr Surg. 2001;10:132-139.
2. 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.
3. 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.
4. 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.

I. 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. Not surprisingly, amplification is associated with undifferentiated and poorly differentiated neuroblastomas with a high mitotic-karyorrhectic index.[1]

MYCN overexpression usually occurs by gene amplification in 1 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 required 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 that of chromosome 2 signals (MYCN gain), is yet to be determined.

Recent studies have identified a subset of neuroblastic tumors with “discordance” between the genotype (MYCN amplification status) and the phenotype (differentiation, MKI, and histologic classification).2 In cases with amplification of the MYCN gene but favorable histologic features (differentiating neuroblasts and/or low-intermediate MKI), the cells do not produce active N-myc protein and lack the classic “bulls eye” nucleoli.[2] In cases that lack MYCN amplification but have unfavorable histologic features (undifferentiated neuroblasts and/or high MKI), C-myc protein is often being expressed instead.[3]

MYCN amplification is also correlated with advanced-stage tumors often having chromosome 1p deletions, especially del 1p36.3.[4] The deletion of 14q has also been shown to be unfavorable, as have loss of 11q and gain of 17q.[5]

ALK Mutation and Amplification

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.[6,7,8] About 8% to 10% of tumors have ALK mutations, and about 25% have gene amplification or protein overexpression; these aberrations are all associated with higher risk and worse

prognosis.[9] Although ALK immunohistochemistry does not always correlate with expression status, gene sequencing (especially of the kinase regions and mutational hotspots) is sometimes performed in treatment-refractory patients who might be candidates for tyrosine kinase inhibitors.

ATRX

Although mutations in the alpha-thalassemia/mental retardation X-linked syndrome (ATRX) gene are only found in 2%-3% of all neuroblastic tumors, the vast majority of high-stage tumors in older children and adolescents have ATRX mutations (whereas congenital and infantile tumors only exceedingly rarely have them).[9,10] The ATRX gene product plays a role in telomere maintenance, and tumor cells with mutated ATRX have longer-than-usual telomeres, prolonging their survival.

DNA Index

Determination of DNA index by flow cytometry is also important; however, a minimum of 100 mg and preferably 1g 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.[5]

Others

Comparative genomic hybridization is typically used to evaluate for segmental chromosomal aberrations (especially 1p deletion, 11q deletion, and/or 17q gain), which are associated with high-risk tumors, whereas alterations in the numbers of whole chromosomes are associated with lower risk tumors.[11,12]

Additional genetic abnormalities may 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.[13] PHOX2B mutations are frequently seen in familial neuroblastomas, but only rarely in sporadic tumors.[14]

References

1. 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.
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