Computer Assisted Isolation of Cancerous cell from Neuroblastoma Histology using Image Segmentation Methods

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Abstract:
Neuroblastoma is the third most common type of childhood cancer in the pediatric age group. It is a type of cancer that is found in the adrenal glands present above the kidney. It usually affects the particular regions such as stomach, chest, neck, pelvis, and bones. Children are usually diagnosed with Neuroblastoma cancer between the age of 1 to 2 year. It can be discovered by certain imaging techniques. Imaging is one of the crucial techniques in the diagnosis, staging, treatment planning, response evaluation, and follow-up of a Neuroblastoma case. The current prognostic classification of this disease is based in part on the morphological characteristics of the cells as seen in the images stained with H&E (Hematoxylin and eosin). The main goal is to detect the cancer cells from the H&E-Stained images by applying color segmentation, cell extraction, boundarization & major minor axis detection.

Keywords:
H&E-stained images, Neuroblastoma, Segmentation, image analysis, Machine learning, Medical image processing.

I. INTRODUCTION

Neuroblastoma is a type of cancer that is found in the adrenal glands present above the kidney. It usually affects the particular regions such as stomach, chest, neck, pelvis, and bones. Children in the pediatric age are the most typically affected by this type of cancer. The most common cancer in children is neuroblastoma (found usually in children younger than 1 year old). In the United States, each year roughly 700 to 800 new cases of neuroblastoma are diagnosed. For many years, this figure of diagnosis has remained constant. Children are usually diagnosed with Neuroblastoma cancer between the age of 1 to 2 years. It can be discovered by ultrasonography even before birth in a small percentage of cases. It is diagnosed in around 9 out of 10 cases. It is uncommon in children above the age of 10 years. The median age at which a child is diagnosed is 22 months. In the United States, neuroblastoma accounts for 6% of all childhood malignancies.

Pathologists must discover certain morphological traits with microscopic analysis of tumour samples in the current grade evaluations for patients with this disease. Neuroblasts are young, undifferentiated small, spherical sympathetic cells with minimal cytoplasm, dark nuclei, and little inconspicuous nucleoli. Homer- Wright rosetles (a type of pseudorosette in which differentiated tumor cells surround the neuropil) are cellular clusters that occur occasionally and are characteristic with Neuroblastoma. The high urinary levels of the cone of catecholamines, as well as the typical histopathologic features, are used to diagnose it. The Shimada classification (figure 1) and The Paediatric Oncology Group (POG) classification are two histological methods that are routinely used to stratify neuroblastic tumours into risk groups on the basis of histological findings and provide a prognosis. According to POG, NBL which accounts for <50% of the ‘differentiated’ can be further classified into the most immature form i.e the undifferentiated, ‘poorly differentiated’ as the most mature form i.e., the differentiated.

II. THE SHIMADA CLASSIFICATION

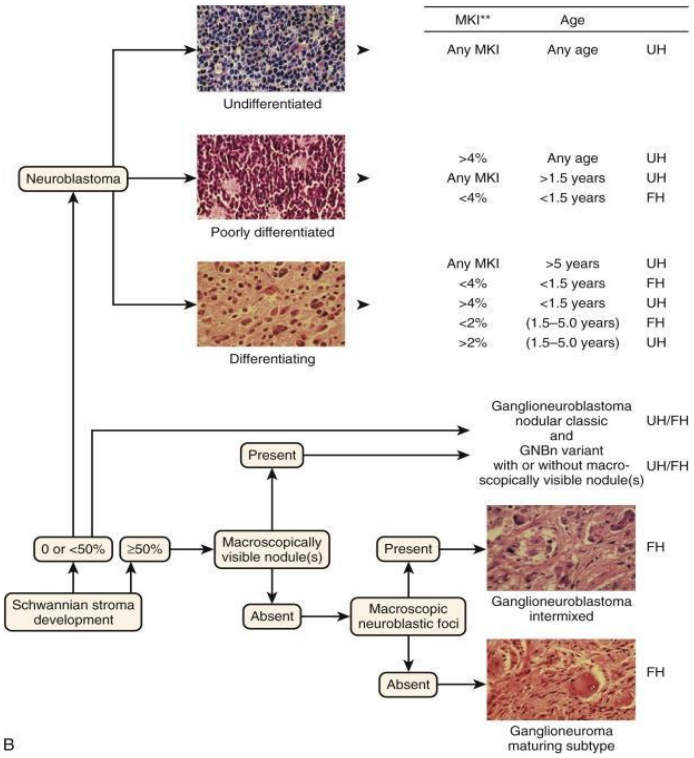

Figure 1. Shimada System: describing how NBL is classified between favorable and un-favorable histology.

It takes into account histological morphologic aspects as well as the patient's age at the time of diagnosis. The combination of patient age, mitosis-karyokinesis index (MKI), cellular and stromal maturity determines whether a cancer is in 'favourable' or 'unfavourable' form.

As we can see in Figure 1. NBL is classified between Favourable and Unfavourable histology characteristics.

Favouorable Histological characteristics
The children of age 1.5-5 years old with a low MKI differentiating or <1.5 years of age with low or intermediate MKI & differentiating or partially differentiating tumour.

Unfavourable Histological characteristics
All other combinations come under the unfavorable histological category.

The treatment is dependent on the age of child, disease stage, risk category & tumour location. The risk categories are as follows:

- Low Risk Neuroblastoma- Children require treatment right away. If the child is younger than 6 months, this tumour does not need treatment and goes away without any treatment.
- Intermediate Risk Neuroblastoma- Children required to undergo a surgery to remove the cancerous cell or tumour.
- High Risk Neuroblastoma- The treatment for this includes the combination of chemotherapy, surgery, radiation, high-dose chemotherapy with stem cell rescue and immunotherapy.

Shimada classification is vital because:

Shimada classification is important because it established a histopathologic classification for patients with neuroblastoma. Around 295 neuroblastoma patients are treated by the Children's Cancer Group, this classification method was examined and linked with outcome (CCG). The following are some of the classification's key features:

- The age of the patient.
- The degree of differentiation of neuroblasts.
- Schwannian stromal development.
- The MKI(Mitosis-karyorrhexis index).
- The Nodular Patterns.

Favorable histology group includes the following:

- Patients of any age who have stroma-rich tumours with no nodular pattern.
- Patients <18 months with stroma-poor tumors, an MKI <200/5000 (200 karyorrhectic cells / 5000 cells which are scanned), and neuroblasts i.e. differentiated or undifferentiated.
- Patients <60 months with stroma-poor tumors, an MKI <100/5000, and well-differentiated tumor cells.

Unfavorable histology group includes the following:

- Patients of any age with stroma-rich tumours and a nodular pattern;
- Patients of any age with stroma-poor tumours, undifferentiated or differentiated neuroblasts, and MKI greater than 200/5000.
- Patients over the age of 18 months with stroma-poor tumours, undifferentiated neuroblasts, and MKIs greater than 100/5000.
- Patients over the age of 18 with stroma-poor tumours, differentiated neuroblasts, and an MKI of 100-200/5000.
- Patients over the age of 60 months with stroma-poor, differentiated neuroblasts and an MKI < 100.

How doctor does the prognosis:

To diagnose neuroblastoma, doctor prognosis involves a physical and neurological examination. A neurological exam is done to check the child's nerve function, coordination and reflexes. For the accurate prognosis

specialist suggest several tests to confirm a diagnosis and check if the cancer has spread and what treatment it may require according to the risk categories.

how computer assisted system are used to classify images:

Early cancer identification can improve patients' chances of survival and treatment options. For cancer diagnosis, medical pictures such as mammograms, ultrasounds, magnetic resonance imaging, and microscopic images are commonly used. Computer-aided diagnosis (CAD) systems have recently been used to assist physicians in cancer diagnosis in order to improve diagnosis accuracy. CAD can aid in the reduction of missed cancer lesions due to physician tiredness, the reduction of workload and data overloading, and the reduction of image reader variability. A framework of CAD systems for cancer diagnosis based on medical pictures was proposed in this study. The suggested study assists clinicians in detecting suspicious regions utilizing several medical picture modalities and identifying such regions as normal or abnormal.

Data Set collection and software and hardware used :

Data Sets were collected from - Image bank, American society of hematology. Image dataset used consists of 36 NBL cases representing all neuroblastic grading subtypes. All the images are 40X resolution and stained with H&E Staining process. MATLAB is used to create the developed classification algorithm and the graphical user interface (The MathWorks, Inc., Natick, MA). Windows 10 home premium is used as an operating system with i3 Intel Processor.

This paper is organized as follows: In section II, segmentation and classification process of previous studies is discussed. Abstract view of proposed system along with data collection procedure is discussed in section III. We have introduced a two-stage binary classification procedure of H&E-stained FL images followed by classification procedures as elaborated in section VI. Methodologies undergone are placed in section V. Experimental results of multistage are placed in section VI. The summarization and conclusive remarks have discoursed in Section VII.

III. SEGMENTATION & ANALYSIS OF NEUROBLASTOMA

Significant efforts have been made to develop a risk-classification algorithm for patients with newly diagnosed neuroblastoma. Most collaborative groups employ a system that combines the evaluation of easily measured clinical variables, such as the patient's age and tumour stage, with the evaluation of specific biologic variables. In that younger patients are more likely to have tumours with biologic features associated with a benign clinical course, the age at diagnosis is considered a surrogate for underlying biologic characteristics. Although age is a continuous variable in prognosis, a cutoff point of 12 or 18 months of age has been used for clinical purposes. Infants with localised tumours are almost always cured, and this is often done without the use of cytotoxic therapy. However, due to the relative rarity of the condition and the evolving nature of molecular diagnostics, it has been difficult to reach a conclusion for patients who fall between these extremes.

IV. PROPOSED METHODOLOGY

An automated grading system is used in this study to perform a quantitative analysis of histological images of H&E-stained NB cross-sections. To mimic the way pathologists examine resected material, the entire image analysis pipeline is built in a multi-resolution paradigm. The developed method works on photos with the lowest possible resolution while retaining enough image features for grade analysis. Important statistical characteristics required for accurate evaluations are constructed from segmented image regions containing the most discriminating information at each level of the image hierarchy.

The systematic selection of the best subset of features improves both of the following system efficiency and grading accuracy. Furthermore, a variety of classifiers are used to explore different sections of the feature space, simulating a prognostic panel with multiple pathologists. The competitive classification accuracy and high throughput performance of the developed system indicate that it has the potential for NB grading assessments.

It is critical to emphasize that a computer-assisted grading system should never be used in place of a pathologist. Instead, it should only be used to aid in decision-making. If the electronic system and the doctor ratings disagree, the human doctors make the final decision (pathologists).
The attempt to identify and localize the compounds that make up the components of living cells and tissues has been one of the key routes toward understanding shape and function. This method's goal was to determine the relationship between substance and structure. One of the most sensitive methods for achieving this goal is the labeled-antibody technique. An antibody is made from a pure cell component, labeled with a marker, and applied to a slice of tissue as a stain in its most basic form. The labeled-antibody approach, on the other hand, entails more than just the synthesis and application of an antibody; it also requires

the preparation of tissue to be stained with the antibody, which must adhere to a set of strict guidelines.

Figure 2 depicts the flow graph i.e. the steps undergone.

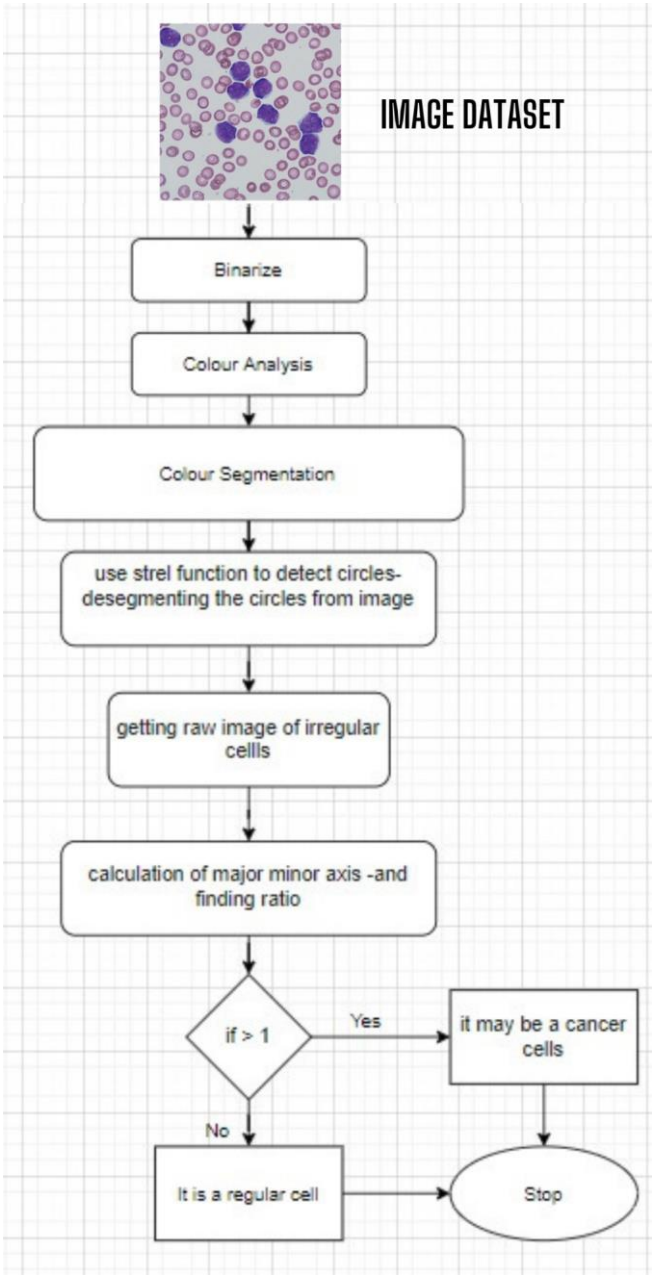

Figure 2. Flowchart of the developed classification system

V. NEUROBLASTIC METHODOLOGIES

- **Image Dataset**

All tumour slides used in the image dataset method are chosen so that they are good representations of different grade subtypes and have a sufficient number of cytological components of interest in the tissue regions. MATLAB is used to create the developed classification algorithm and the graphical user interface (The MathWorks, Inc., Natick, MA). The image dataset used consists of 36 NB cases representing all neuroblastic grading subtypes.

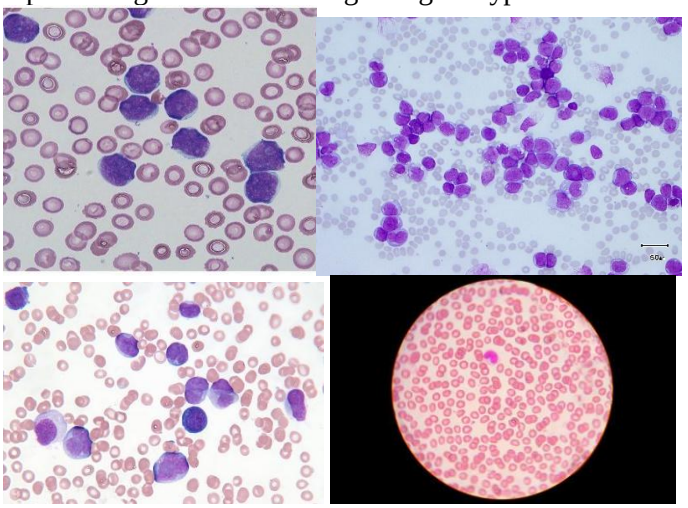

Figure 3. Datasets collected (H&E stained images)

- **Image Acquisition**

During the preparation stage of the image acquisition method the tissue slides are sliced at a thickness of around 5µm and then are soaked in paraffin. The dual staining method is used to stain each neuroblastoma slide in which Hematoxylin & eosin(H&E) are used so that visual contrasts of different cytological components can be increased. After the image is being stained by the dual staining method, each tissue slide prepared is placed on a scanning bed and is then digitized with the help of Scan Scope T2 digitizer, allowing for clear visualization of tumour architectures.

- **Color Segmentation**

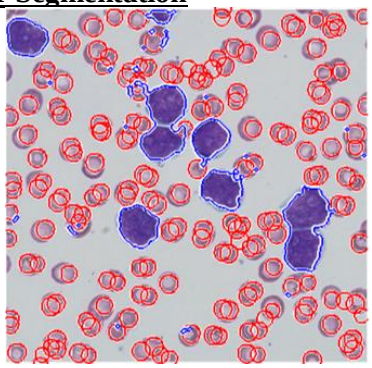

Figure 4. Segmented image wherein the red outlined cells depict the regular and blue outlined cells depict the irregular cells.

Steps followed are :-

1. Read original image -

The original image is taken as an input by the function "imread' function further we've done various color varieties for example further we've turned the rgb image to the gray scale image .

The RGB2GRAY function is used for converting the image to the gray scale for better recognition of the cells.

The cells can be easily differentiated for better further understanding of the concept.

2. Convert into grayscale -

Original image is converted into grayscale using RGB to gray.

3. Red-blue cell separate -

These images have been taken for further detection of the boundaries of blue channel or red channel separately.

```
red=img(:,:,1)
subplot(4,2,3)
blue=img(:,:,3)
subplot(4,2,4)
```

4. Extract irregular cell -

Irregular cells are detected as they are not at all fully rounded . They are detected by the strel function that is clearly explained in the matlab function.

5. Extract regular cell -

Regulate cells means perfectly round cells that are present in the cells and are more easy to get extracted

they are also get extracted from the strel function by using disc and assigning the radius as a parameters for further detection of boundaries

6. Add both images & invert the output -

The images further we're working upon are binarized and the black and white are further converted on the white and black that is the perfectly opposite side so that the regulate cells can be separated from the irregular cells .This strategy has helped us for the separation of the regular cells from the irregular cells through 'imbinarize' function only.

7. Count the circle -

Now the circles that were as seen in Figure 4. detected whether regular or irregular are to be counted for further detection and calculation purposes. The count of red cells, that is the normal cells will always be larger than the number of irregular cells because the irregular cells are basically the cancer cells and the regular ones.The main aim for the project is to detect the cancer cells and conclude whether the person is infected or not. So this can occur basically from the image of the tissue cells only.

- **Boundarization of irregular & regular cells**

Boundarization means the cells are boundariesed as to detect whether they are regular or irregular as it plays important role in the detection of the cancer tissues.

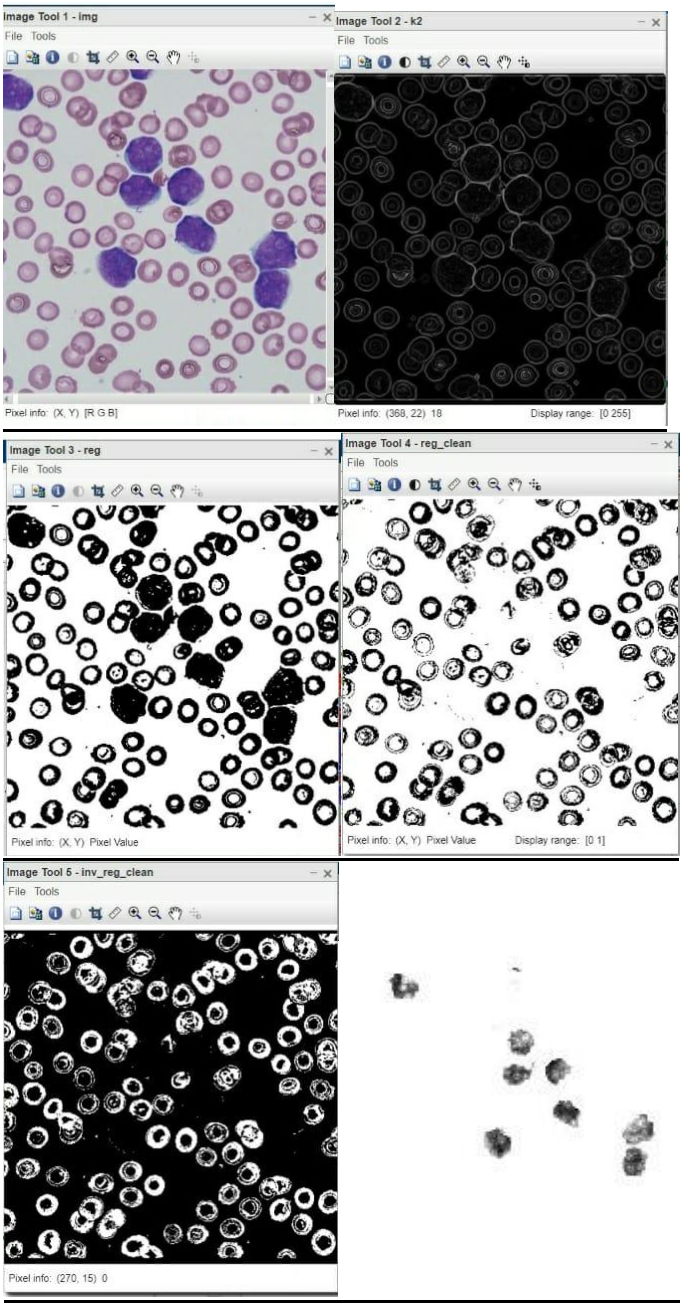

Figure 5. The images acquired after the boundarization

Here the H&E stained images were taken as an input and the cells were in red and in blue color . So methodologies were applied in order to detect the blue color cells that are the rounded cells and the red cells were actually the irregular cells which are hence the cancer cells. For Clarity

purposes , we made the image turn into the black and white from the RGB part which can be observed in the Figure 5.

Steps taken for Boundarization are,

- The image is taken as the input
- The image is converted from RGB to GRAY
- The structuring element function "STREL" function is taken where we define parameters as:
  Disc
  Diamond
  Octagon
  Line
  Triangle
- 'imdilate' function dilates the image it dilates the grayscale, binary, or packed binary image I using the structuring element SE
- And for displaying the original image 'imtool' function is used at the end

- **Major minor axis**

The major axis as seen in Figure 6. is the line segment that connects the two most distant points on an ellipse. The minor axis as seen in Figure 6 is the one that passes through the closest point. Firstly we have taken the output image of the boundarization of irregular cells as our input. The image is then preprocessed, then noise removal filtering is complete.

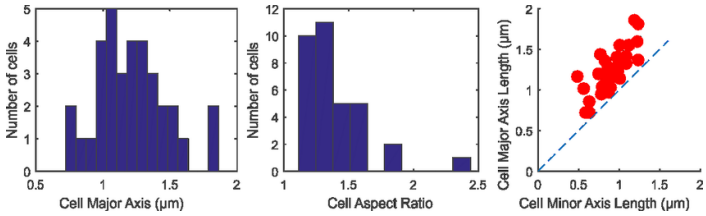

Figure 6. graph depicting the calculation of the major minor axis

The dotted line has an aspect ratio of one, indicating that many cells are elongated.

- **Cancer Detection**

Early detection of cancer is one of the most difficult problems in cancer treatment. Cancer is frequently discovered in its advanced stages, when it has compromised the function of one or more vital organ systems and has spread throughout the body. Methods for early detection of cancer are critical, and they are a focus of current research.

VI. RESULT

Before the developed system can do an inexpensive level of grading accuracy, it must be thoroughly trained. A high-level overview of the training–testing process is depicted in, with solid and dashed arrows indicating the steps performed online and offline, respectively. Because the ADP system is trained with more and more typical patterns, it begins to supply outputs that resemble those of the pathologist. Following the completion of the training process, testing data is passed into the "educated" system for evaluation of its performance. The evolving system could be a valuable tool that pathologists and clinicians can use to see the grade of NB. Although the computerized system's decision accuracy was promising in our tests, it should be noted that the computerized system's role must always be limited to that of a second reader or pre-screener. In other words, the computerized methodology isn't meant to exchange the pathologist, but rather to supplement the quality prognostic procedure. Because neuroblastic differentiation is critical for correct protocol assignment, we'll still improve the developed system in our future work. Because higher-order decision information can contribute to either a more efficient or more accurate global labeling configuration, it's preferable to form a labeling scheme that comes with decision information from adjacent image tiles. Expansion into a broader set of features is another area of research that we are going to investigate within the future, as our current implementation only uses color and texture information. We are also going to check out a way to group the simplest training datasets, as data generalization could be a critical issue that may have a big impact on overall performance.With all of those aspects improved, it's reasonable to anticipate even better classification accuracy adequate for clinical requirements in practice.

VII. CONCLUSION

The entire image analysis pipeline is designed to work in a multi-resolution environment. The system's categorization accuracy is 87.88 percent. The created method chooses to work on photos with low resolution levels that maintain enough image features for grading analysis. For examining different sections of the feature space, a series of classifiers is used, simulating the situation when many pathologists are present within a prognostic panel.
An international collaboration is currently underway to assess the clinical and biologic characteristics of neuroblastic tumours (NTs) (a group of tumours that includes neuroblastoma, ganglioneuroblastoma, and ganglioneuroma) in order to develop treatment strategies

based on a comprehensive set of International Neuroblastoma Risk Groups. A global Neuroblastoma Staging System and a set of International Neuroblastoma Response Criteria have been developed. As a result of the international collaboration to date, a global Neuroblastoma Staging System and a set of International Neuroblastoma Response Criteria have been developed. The International Neuroblastoma Pathology Committee (INPC), established in 1994, has been actively participating in this endeavor by proposing a global pathology classification.

Because of their "unexpected" clinical traits, such as involution/spontaneous regression, maturation, and aggressive progression, many oncologists and researchers initially thought of NTs as "enigmatic." Clinical and basic research discoveries have accumulated data that allows us to elucidate NTs in a whole new way (i.e., NTs are "heterogeneous," with their individual biologic features intimately tied to their unique clinical behaviour). Long regarded to be the most essential morphologic feature for NT prognosis, the degree of neuroblastic maturation toward ganglion cells Over the years, several histopathologic grading systems for NTs have been devised, but none has gained universal approval and application to our knowledge. Shimada's age-linked categorization, which was a first, divided NTs into Schwannian stroma-rich and stroma-poor tumours. To explain one of the prognostic markers, they established the term "mitosis-karyorrhexis index" (MKI). Joshi et al. modified the present categorization, claiming that a high mitotic rate is an unfavourable prognosis factor, whereas tumor-associated calcification is a prognostic factor. Neuroblastic tumours have crucial biological traits that have influenced our understanding during the last decade. Those tumours are neoplasms. Any illness classification system, like others before it, has the challenge of developing a repeatable and biologically relevant system.

This paper summarizes the INPC's four-year operations, which were supported by the key international partnership on NTs:

- Creating consensus diagnoses to support consistent morphologic feature criteria, and
- Using the consensus diagnoses to assess the prognostic importance of the morphologic characteristics and their combinations.

The INPC's mission is to provide a global Neuroblastoma Pathology Classification that is prognostically useful, physiologically relevant, highly reproducible, and user-friendly. The exact criteria for morphologic features of the NTs used in this study were published in a prior work. Within the current analysis, we also provide a recommendation/guideline for surgical pathologists to follow in describing and prognosticating NTs.

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