Multinational Retrospective Central Pathology Review of Neuroblastoma

Lessons Learned to Establish a Regional Pathology Referral Center in Resource-Limited Settings

Teresa Santiago, MD; Ana C. Polanco, MD; Soad Fuentes-Alabi, MD; Caleb Hayes, MPH, MA; Elizabeth Orellana, MD; Belkis Gomero, MD; Mázlova Toledo González, MD; Eduviges Ruiz, MD; Moisés Espino Durán, MD; Carlos Rodríguez-Galindo, MD; Monika Metzger, MD.

From the Departments of Pathology (Santiago, Hayes) and Global Pediatric Medicine (Rodriguez-Galindo, Metzger), St Jude Children's Research Hospital, Memphis, Tennessee; the Departments of Pathology (Polanco) and Oncology (Fuentes-Alabi), Hospital Nacional de Niños Benjamín Bloom, San Salvador, El Salvador; the Department of Pathology, Francisco Marroquín Medical School, Guatemala City, Guatemala (Orellana); the Department of Pathology, Hospital Infantil Dr Robert Reid Cabral, Santo Domingo, Dominican Republic (Gomero); the Department of Pathology, Hospital Escuela-Universitario, Tegucigalpa, Honduras (González); the Department of Pathology, Hospital Infantil Manuel de Jesus Rivera, Managua, Nicaragua (Ruiz); and the Department of Pathology, Hospital del Niño Dr. José Renán Esquivel, Panamá, Panama (Durán).

The authors have no relevant financial interest in the products or companies described in this article. Corresponding author: Teresa Santiago, MD, Department of Pathology, St Jude Children's Research Hospital, 262 Danny Thomas Place, MS 250, Memphis, TN 38105-3678 (email: *Teresa.Santiago@stjude.org*).

Accepted for publication April 2, 2020.

Published online June 3, 2020.

Context.—Several countries of the Central America and Caribbean region have been sharing regional neuroblastoma (NB) treatment guidelines. However, there is no standardization in the diagnosis, subclassification, or tumor biology to aid in the risk stratification of these patients.

Objective.—To examine the histology and assess the accuracy of the local pathology reports; to evaluate the usefulness of manual MYCN immunohistochemistry (IHC); and to use NB as a model to identify the needs to establish a central pathology review (CPR) program in this region.

Design.—A retrospective CPR of specimens derived from patients with a diagnosis of NB and treated under the regional NB guidelines between 2012 and 2017 was conducted, allowing for a comparison between local diagnoses and the CPR diagnoses. Manual MYCN IHC was performed in the confirmed NB specimens and the results compared with known fluorescence in situ hybridization or automated IHC results, when available.

Results.—The 156 specimens reviewed included 460 blocks and 183 original slides. Neuroblastoma was confirmed in 138 samples (88.5%), but low concordance rates for Shimada classification (n = 39; 25.0%), mitotickaryorrhectic index (n = 4; 2.5%), and International Neuroblastoma Pathology Classification (n = 18; 11.5%) were noted. Manual MYCN IHC performed on 120 specimens showed conclusive results in 89.2% (28 positive, 23.4%; 79 negative, 65.8%) and questionable results in 10.8% (n = 13).

Conclusions.—This retrospective CPR highlights the need for a CPR program to serve this region, to ensure correct diagnosis and subclassification of NB, and to provide manual MYCN IHC—with reflexing to fluorescence in situ hybridization, if questionable. This approach can further regional collaboration, enhance test utilization, and ultimately improve patients' outcomes.

(Arch Pathol Lab Med. 2021;145:214–221. Doi: 10.5858/arpa.2019-0570-OA).

Established in 1998, the Asociación de Hemato-Oncología Pediátrica de Centro América (AHOPCA) is a consortium of pediatric oncology centers from Central American and Caribbean region countries, including Nicaragua, Guatemala, El Salvador, Honduras, Costa Rica, Panamá, Haiti, and the Dominican Republic. The AHOPCA group has developed shared clinical guidelines and adopted cooperative regional pediatric oncology treatment protocols to advance the care provided and improve the survival of children with cancer from these countries. Although the overall outcome of children with cancer from the AHOPCA region has improved over time because of advances in local clinical capacity, improvements in supportive care, and the use of adapted multicenter regional standardized clinical guidelines,1–4 there is still no uniform dependability in the pathologic diagnosis in this region. Some centers have excellent pathology diagnostic capability, whereas others still struggle with many constraints. Moreover, adequate histologic subclassification of specific tumor types, such as neuroblastoma (NB), that can impact risk stratification and ultimately affect treatment and outcomes is not always provided.

When clinicians or pathologists from the AHOPCA region are confronted with challenging cases, they traditionally seek out expert second opinion outside the AHOPCA region.5 However, in the last few years we have been working to uniformly improve the quality of the local pathology service provided in this limited-resource area.6 We have encouraged collaboration among the AHOPCA pathology laboratories to optimize resource use and to eventually become a self-sufficient pathology regional network that can deliver adequate diagnostic support to the clinical services in the AHOPCA countries.

For many years centralized pathology review by expert pathologists has been successfully used as a strategy for quality assurance and to improve diagnostic accuracy. Therefore, we sought to perform a centralized retrospective pathology review of a cohort of patients with a diagnosis of NB and treated under a regional NB guideline (AHOPCANB2012) with the main objectives of: (1) examining the

histologic characteristics of specimens derived from this cohort of patients and assessing how accurate the information provided in the original pathology report was; (2) evaluating the usefulness of performing MYCN immunohistochemistry (IHC) manually in a region with limited resources; (3) using NB as a model to identify the needs for the implementation of an independent and successful central pathology review (CPR) program to serve the AHOPCA region. Such a CPR program can ensure the correct pathology diagnosis, provide more accurate information for treatment guideline data analysis, and in some instances may influence the treatment selection (if performed prospectively as rapid central review), with the potential to impact patients' outcomes.

The risk assignment and treatment selection of patients with NB are significantly affected by proper morphologic classification (Modified Shimada Classification and the International Neuroblastoma Pathology Classification [INPC]),7,8 and by the determination of some biologic features, such as MYCN status.9 Thus, in addition to verifying the original diagnosis and its proper morphologic classification and subclassification, we sought to investigate the feasibility for a CPR center in the AHOPCA region to perform any additional assays that are necessary for the adequate risk stratification of these patients with NB, such as verification of MYCN status.

Here, we describe a retrospective CPR of samples collected from patients diagnosed as having NB and treated according to a multicenter regional treatment guideline in countries with limited resources. A subset of these patients had an external second opinion at the time of the initial diagnosis with known MYCN status. The discrepancies and similarities in the histologic evaluation between the original (local) diagnosis and CPR diagnosis will be emphasized. A feasibility analysis of using MYCN IHC performed by hand (manual) at the CPR center as a surrogate for fluorescence in situ hybridization (FISH) to investigate MYCN status in patients with NB will also be discussed.

Materials and methods

Cohort

After approval by the Institutional Review Board from all the participating institutions, a retrospective search into the Pediatric Oncology Network Database (POND; www.pond4kids.org; accessed March 31, 2020)10 was done to identify all the pediatric patients with a diagnosis of NB in any of the AHOPCA countries between 2012 and 2017 who were treated according to the AHOPCA-NB2012 guidelines. Necessary relevant clinical information, such as age at diagnosis and patient sex, was obtained from the POND database when not available in the original pathology report. We requested all the contributing institutions to submit to the designated CPR center via certified mail a copy of the original pathology report, original hematoxylin-eosin (H&E)–stained slide(s), and at least 1 representative formalin-fixed, paraffinembedded tissue block from all available specimen(s) of each patient with a diagnosis of NB. A further search was performed in our pathology consultation database to investigate if any of these cases had also been submitted to our institution for a second opinion.

The CPR Center and the Review Process

The Department of Pathology of the Hospital Nacional de Niños Benjamín Bloom (HNNBB) in San Salvador, El Salvador, was chosen to be the CPR center for this retrospective review. This is a well-equipped institutional anatomic pathology laboratory that has consistently demonstrated an overall excellent technical quality and has manual IHC assay available.6 The cases submitted for central review were first assessed by the HNNBB pathologist (A.P.), who selected the best provided block to be used for any additional IHC (including MYCN immunostain). At least 1 block per case was recut and stained with H&E at HNNBB. If needed, other IHC stains were performed to confirm or to rule out the diagnosis of NB. The final CPR was conducted during 7 days by 2 pathologists (a senior general pathologist from HNNBB with more than 20 years of experience and

a board-certified pediatric pathologist with expertise in pediatric oncology), jointly using a multihead microscope, who were blinded to the original diagnosis or any previous second opinion diagnosis, if available. When the originalH&E-stained slides were submitted for review, an overall quality evaluation considering fixation, processing, quality of the tissue section, and quality of the staining was appraised, and the specimens were scored as being of excellent, acceptable, poor, or inadequate quality. In all confirmed NB cases, the Mitotic-Karyorrhectic Index (MKI) and the INPC were assigned, if applicable, and then MYCN immunostaining was performed. If more than 1 specimen was submitted from the same patient (eg, initial biopsy and posttherapy sample), at least 1 sample per patient was stained with MYCN IHC—always giving preference to the specimen that had more representative lesional tissue available.

MYCN Immunohistochemistry

Currently, the IHC menu at HHNBB includes 66 antibodies. MYCN antibody was not available at HNNBB before this CPR. A rigorous validation and optimization process using a mouse monoclonal MYCN antibody (clone NCM II 100, Abcam ab16898) was performed to determine a final optimal dilution of 1:200. A tissue microarray obtained from a cohort of NB cases with known MYCN status by FISH and protein expression level by automated IHC (10 positive cases and 10 negative cases) were used for validation.11 All the steps of the manual MYCN staining performed in this study were done following the manual IHC protocol currently in use at HNNBB, as previously described.6 Nuclear positivity was interpreted as evidence of MYCN protein expression; conversely, the absence of nuclear staining was scored as negative. For any case in which the positivity or lack thereof was not convincing, the specimen was categorized as having a questionable MYCN IHC result.

Data Collection and Analysis

The collected data for this study included information obtained from the original pathology reports and the information captured

during the CPR. A comparison between the initial local diagnosis, MKI, and INPC specified in the provided pathology report, and final CPR diagnosis, MKI, and INPC, was done. If the MKI or INPC was not included in the original pathology report, it was recorded as information not provided.

The results of the MYCN IHC performed during the CPR in the confirmed NB cases were noted as positive, negative, questionable, or not performed. A subset of the samples had previously been submitted for a second opinion and had known MYCN gene status by FISH and/or MYCN protein expression level determined by automated IHC, which allowed a comparison between automated versus manual MYCN IHC results performed at HNNBB.

Table 1. *Material Submitted for Central Pathology Review of Neuroblastoma by Location*

City/Country	Total No. (%) of Cases in the AHOPCA NB2012 between 2012 and 2017	Total No. (%) of Patients Not Reviewed	Total No. (%) of Patients Reviewed	Total No. (%) Enrolled of Specimens Reviewed
Guatemala City/ Guatemala	35 of 189 (18.5)	7 of 35 (20.0)	28 of 35 (80.0)	35 of 156 (22.4)
Managua/ Nicaragua	38 of 189 (20.1)	6 of 38 (15.8)	32 of 38 (84.2)	36 of 156 (23.1)
Panama City/ Panama	12 of 189 (6.4)	3 of 12 (25.0)	9 of 12 (75.0)	10 of 156 (6.4)
San Pedro Sula/ Honduras	22 of 189 (11.7)	15 of 22 (68.2)	7 of 22 (31.8)	7 of 156 (4.5)
San Salvador/ El Salvador	39 of 189 (20.6)	9 of 39 (23.1)	30 of 39 (76.9)	44 of 156 (28.2)
Santo Domingo/ Dominican Republic	29 of 189 (15.3)	16 of 29 (55.2)	13 of 29 (44.8)	17 of 156 (10.9)
Tegucigalpa/ Honduras	14 of 189 (7.4)	8 of 14 (57.1)	6 of 14 (42.9)	7 of 156 (4.5)
Total	189 of 189 (100.0)	64 of 189 (33.9)	125 of 189 (66.1)	156 of 156 (100.0)

Results

Material Submitted for CPR

We received and reviewed specimens from 125 of 189 patients treated according to the AHOPCA NB2012 guidelines between 2012 and 2017, which represents specimens from 66.1% of the entire cohort. The reasons for the exclusion of 64 cases (33.9%) were: insufficient material available for review, including missing both the block(s) and original pathology report (41 of 64; 64.1%); only the paraffin block(s) was unavailable (13 of 64; 20.3%); the original pathology report was unobtainable (1 of 64; 1.5%); or other nonspecified reasons (9 of 64; 14.1%; Table 1). A total of 156 specimens from 125 patients were analyzed. We reviewed a single sample from 97 patients, 2 samples from 25 patients, and 3 distinct specimens from 3 patients. The materials were submitted from 7 pediatric oncology centers from the AHOPCA region and included 460 paraffin blocks and 183 original H&E-stained slides. The average number of blocks and slides per specimen were 2.9 blocks (range, 0–21) and 1.1 slides (range, 0–16). Most of the specimens examined were from El Salvador (44 of 156; 28.2%), Nicaragua (36 of 156; 23.1%), and Guatemala (35 of 156; 22.4%). The specific number of specimens, the number of submitted blocks, and slides for the CPR per participating centers are outlined in Table 2.

Clinical Characteristics and Quality of the Submitted Material

The specimens (n ¼=156) submitted for review were obtained from 125 patients: 53 female patients (42.4%) and 72 male patients (57.6%). The mean age at diagnosis was 39.8 months (ranging from 1 month to 15.5 years), with 39 patients younger than 18 months (31.2%), 60 patients with age 18 months to less than 5 years (48.0%), and 26 patients older than 5 years (20.8%) at the time of diagnosis.

The types of procedures performed to obtain the samples were specified in 69.2% of the submitted pathology reports (108 of 156) and included 48 resection specimens (30.8%), 47 incisional biopsies

(30.1%), and 13 core needle biopsies (8.3%). In 48 of 156 samples, the type of procedure was not stated (30.8%). Information indicating that the patient had received preoperative chemotherapy was specified in 17.3% of the submitted pathology reports (27 of 156).

Similarities and Divergences in the Diagnosis and Classification of NB

After light microscopic examination, a final CPR diagnosis was provided for 91.1% of the submitted material (142 of 156), and the diagnosis of NB was confirmed in 138 specimens (138 of 156; 88.5%). The 14 specimens in which a final definitive diagnosis could not be reached included: 3 specimens classified as malignant tumor not otherwise specified (3 of 156; 1.9%); 9 samples in which tumor was not seen (9 of 156; 5.8%); 1 where the material was considered inadequate for morphologic evaluation (1 of 156; 0.6%); and an additional case in which wrong blocks and slides (with a different accession number from the submitted pathology report) were provided for the CPR (1 of 156; 0.6%).

Slightly more than half of the cases were classified at the CPR as NB, Schwannian stroma poor, and poorly differentiated (74 of 142; 52.1%), and the second most common diagnosis was posttherapy NB (25 of 142; 17.6%). Fourteen specimens had a final diagnosis of NB, Schwannian stroma poor, undifferentiated at the CPR (14 of 142; 9.9%)—in all of them, the diagnosis was confirmed with IHC (including positivity for synaptophysin). Other CPR diagnoses included 5 NB, Schwannian stroma poor, differentiating (5 of 142; 3.5%), 13 ganglioneuroblastoma intermixed (13 of 142; 9.2%), 1 ganglioneuroblastoma nodular (1 of 142; 0.7%), and 6 cases of NB not otherwise specified (6 of 142; 4.2%). The 4 cases in which the diagnosis of NB was not confirmed (4 of 142; 2.8%) included 1 adrenal cortical neoplasm (positive for inhibin, Melan-A, and cytokeratin, but negative for synaptophysin and chromogranin), 1 pelvic immature teratoma, 1 B-cell lymphoblastic lymphoma (CD45+, CD20+, and TdTþ, but negative for CD3 and synaptophysin), and a case of myeloid sarcoma involving the frontal region

(CD45+, CD43+, MPO+, and CD117+, but negative for CD1a, cytokeratin, desmin, and synaptophysin).

The correlation between the original (local) diagnosis specified in the provided pathology report and the CPR is outlined in Figure 1. Whereas the diagnosis of NB was confirmed in 88.5% of the specimens, a complete agreement between original and final CPR diagnosis with a proper modified Shimada subclassification was observed in only 39 specimens (39 of 156; 25.0%). The similarities and discrepancies between the MKI and the final INPC assigned at the CPR, when applicable, and the MKI and INPC specified in the original diagnosis were also assessed and showed a much lower agreement rate, with 2.5% for MKI (4 of 156) and 11.5% for the INPC (18 of 156; Figure 2).

The 183 original H&E-stained slides received for review were obtained from 80 specimens. The slides were graded as excellent in 10.0% of the specimens (8 of 80), acceptable in 58.8% (47 of 80), poor in 26.2% (21 of 80), and inadequate in 5.0% (4 of 80). The microscopic evaluation at the CPR of the remaining 76 specimens (48.7%) was exclusively based on slides cut and stained at HNNBB (no original slides were provided for review).

Table 2. *Specific Number and Percent of Specimens, Blocks, and Slides Submitted for the Central Pathology Review perParticipating Centers*

City/Country	No. (%) of Specimens Submitted for Review	No. (%) of Blocks Submitted for Review	Average No of Blocks (Range)	No. (%) of Original H&E Slides Submitted for Review No. (%)	Average No of H&E Slides (Range)
Guatemala City/ Guatemala	35 of 156 (22.4)	48 of 460 (10.4)	1.3 (0–6)	48 of 183 (26.2)	1.3 (0–6)
Managua/ Nicaragua	36 of 156 (23.1)	173 of 460 (37.6)	4.8 (1–21)	0 of 183 (0.0)	0.0 (0–0)
Panama City/ Panama	10 of 156 (6.4)	35 of 460 (7.6)	3.5 (1–21)	42 of 183 (23.0)	4.2 (2–8)
San Pedro Sula/Honduras	7 of 156 (4.5)	15 of 460 (3.3)	2.1 (1–8)	0 of 183 (0.0)	0.0 (0–0)

San Salvador/ El Salvador	44 of 156 (28.2)	140 of 460 (30.5)	3.1 (1–6)	53 of 183 (29.0)	1.2 (0–16)
Santo Domingo/ Dominican Republic	17 of 156 (10.9)	30 of 460 (6.5)	1.7 (0–10)	27 of 183 (14.8)	1.5 (0–4)
Tegucigalpa/ Honduras	7 of 156 (4.5)	19 of 460 (4.1)	2.7 (1–5)	13 of 183 (7.0)	1.8 (0–7)
Total	156 of 156 (100.0)	460 of 460 (100.0)	2.9 (0–21)	183 of 183 (100.0)	1.1 (0–16)

Abbreviation: H&E, hematoxylin-eosin staining.

Figure 1. *Multinational retrospective central pathology review of neuroblastoma. Correla- tion between original diagnosis and central pathology review diagnosis. Green area, agreement between original diagnosis and central review diagnosis (n 39 of 156; 25%). *Wrong material submitted for review. †Original diagnoses of "not neuroblastoma": Ewing sarcoma, clear cell sarcoma of kidney, primitive neuroectodermal tumor, adrenal cortical tumor, immature teratoma, ganglio- blastoma, and Wilms tumor; ‡Central review diagnoses of "not neuroblastoma": adrenal cortical tumor, immature teratoma, B-cell lymphoblastic lymphoma, and myeloid sarcoma. Abbreviations: GNB, ganglioneuroblasto- ma; NB, neuroblastoma; NOS, not otherwise specified; SSP, Schwannian stroma poor.*

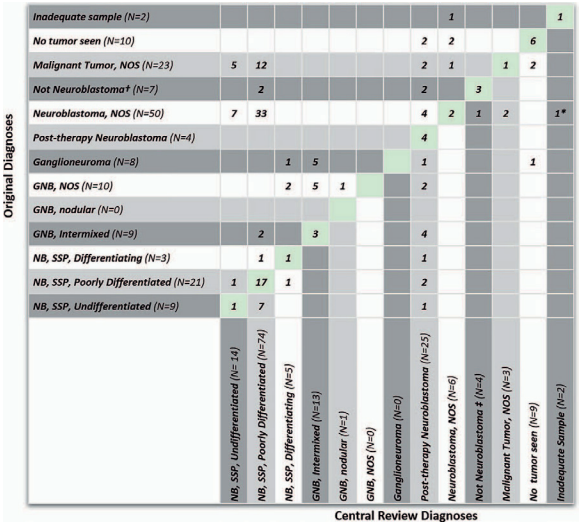


Figure 2. *A, Multinational retrospective central pathology review of neuroblastoma. Correlation between original and central pathology review Mitotic-Karyorrhectic Index (MKI): high MKI, >200 per 5000 cells; >4%; intermediate MKI, 100–200 per 5000 cells; 2%–4%; low MKI,<100 per 5000 cells;<2%. Green area, agreement between original MKI and central review MKI (n¼ 4 of 156; 2.56%). *Reasons why original pathologist could not determine MKI: because the "neoplasia is very undifferentiated." **Reasons why the central review could not determine MKI: not sufficient material, crushing artifacts, bone marrow sample. †Reasons why MKI was not applicable by central review: ganglioneuroblastoma, bone marrow samples, postchemotherapy samples, not NB. ±Original diagnosis others: mitotic index specified in a different manner other than the traditional MKI, such as "10/10 mitoses per HPF, MKI >100, MKI 2/10 HPF, MKI 3/10 HPF," among others. B, Correlation between the original and central pathology review INPC. Green area indicates agreement between original INPC and central review INPC (n ¼ 18 of 156; 11.54%). *Reasons why central review could not determine INPC: because of insufficient material, MKI could not be assessed, or suboptimal sample. +Reasons why INPC was considered not applicable by central review: because of secondary to chemotherapy, not NB, no tumor seen, secondary or tertiary NB sample. Abbreviations: HPF, high-power field; INPC, International Neuroblastoma Pathology Classification; NB, neuroblastoma.*

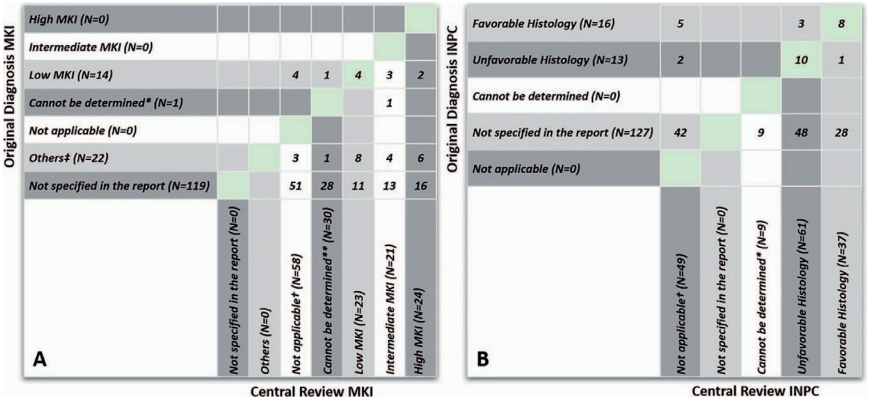
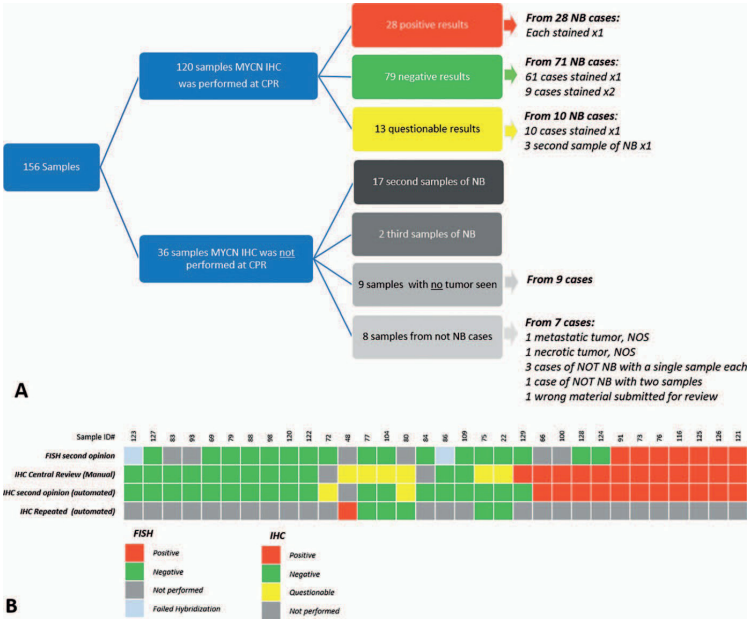


Figure 3. *MYCN immunohistochemistry (IHC) staining. A, Results of the manual MYCN IHC performed at the central pathology review (CPR). B, Correlation between manual MYCN IHC, automated MYCN IHC, and fluorescence in situ hybridization targeting the MYCN gene among 32 neuroblastoma samples. Abbreviations; FISH, fluorescence in situ hybridization; NB, neuroblastoma; NOS, not otherwise specified.*



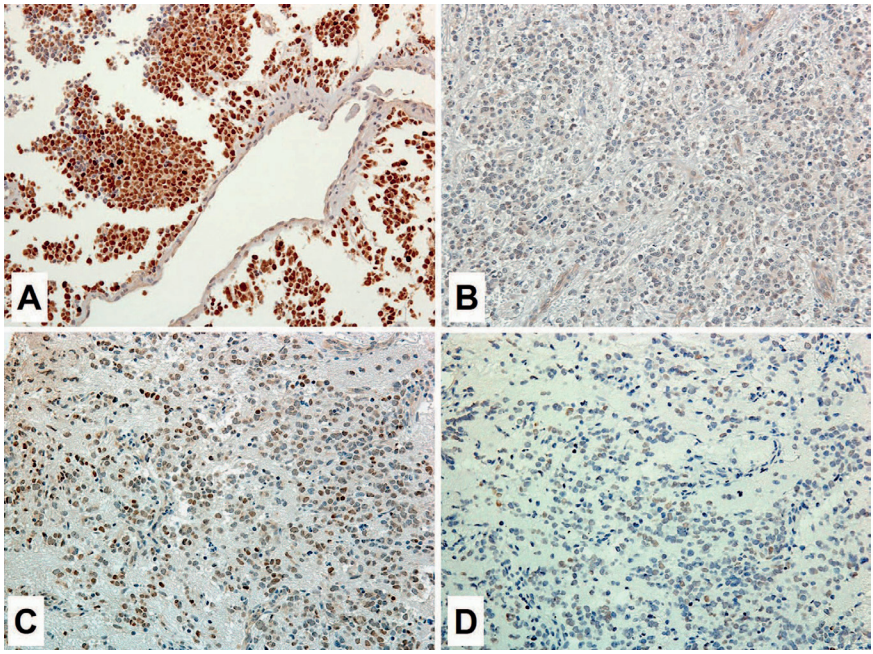
MYCN Protein Expression by Manual IHC and Its Correlation With Automated IHC and FISH

Manual MYCN IHC stain was performed at HNNBB in 120 NB specimens from 109 patients (Figure 3, A). Positive nuclear MYCN protein expression was observed in 28 specimens (from 28 patients of 109; 25.7%), and no evidence of MYCN staining was noted in 79 specimens (from 71 patients of 109; 65.1%). In 13 specimens from 10 cases (10 of 109; 9.2%), the MYCN staining was read as questionable. A total of 11 of 13 questionable manual MYCN IHC results were later repeated with an automated staining procedure, and the results

were compared. A total of 9 samples were rescored as negative (9 of 13; 69.2%), and 2 cases showed focal but unequivocal positivity (2 of 13; 15.4%). In the remaining 2 questionable cases (2 of 13; 15.4%), a repeated automated MYCN staining could not be performed because no more tissue was available in the blocks.

Specimens from a subset of this cohort (from 32 patients) had been previously submitted for pathology second opinion. The MYCN gene status by FISH was available from 26 patients, and automated MYCN IHC stain results were available for comparison from 31 of 32 cases. A parallel evaluation of the results from the 3 distinct assays is presented in Figure 3, B. For instance, all 7 cases that had MYCN gene amplification previously detected by FISH showed positive MYCN protein expression by both manual and automated IHC. Conversely, an additional 8 cases displayed concordant results with negative FISH, and negative manual and automated IHC. Five cases in which FISH study was not initially performed during the previous pathology second opinion consult showed similar results between the automated and manual IHC. Six cases had questionable manual MYCN IHC results, but when performed using an IHC automated stainer, there was no evidence of MYCN protein expression (negative). Examples of positive, negative, and questionable MYCN by IHC in NB cases during the CPR are presented in Figure 4.

Figure 4. *Examples of MYCN immunohistochemistry performed in neuroblastomas during the central pathology review. Positive MYCN result (manual staining procedure) with distinct diffuse and strong nuclear positivity (A); negative MYCN result (manual staining procedure) with mild background staining (1) (B); Questionable MYCN result (manual staining procedure) with mild background staining (1) and equivocal weak nuclear positivity (C); same case as shown in C, MYCN staining performed with an automated stainer interpreted as negative, 200X (D) (original magnification 3200)*



Significant Challenges Faced During the Central Review Process

Even though a border control agreement between El Salvador, Guatemala, Honduras, and Nicaragua (Central America-4) has been in effect since 2006, we unfortunately still encountered a considerable delay in clearing customs within the AHOPCA region. Other challenges faced during this CPR were suboptimal packaging of the submitted material, resulting in broken slides and melted paraffin blocks, problems with the identification of some of the samples submitted, such as unclear labeling, and issues with the original pathology report, including discordant date of birth and age for the patient. Delay with the delivery of reagents at the CPR center by the vendors (eg, delivery of the MYCN antibody) occurred, which not only impacted the projected timeline of this review but also emphasized the everyday challenges that some areas of the world (in particular countries with limited resources) need to endure in dealing with setbacks in receiving reagents and necessary supplies.

Discussion

The practice of performing a CPR for patients enrolled in a multicenter protocol or clinical trial is a well-established procedure and usually includes standardization of the pathologic evaluation. This approach proved to be very influential in the improvements of treatment regimens, inparticular for the treatment of oncologic patients. Some examples in the field of pediatric oncology of very impactful CPR have been demonstrated by the Children's Oncology Group and the International Society of Pediatric Oncology.12–14 Teot et al15 from the Children's Oncology Group examined the past and present use of the CPR in pediatric oncology and emphasized its efficacy and highlighted the applicability of a rapid (prospective) central review. The authors also pointed out the effect that discrepancies between the original diagnosis and the final CPR diagnosis could cause by bringing up concerns regarding the appropriateness of the actions taken before the retrospective pathology review. On the other hand, the usefulness of a retrospective CPR as an assessment tool to investigate the accuracy of the diagnosis and adequacy of the data provided in the pathology report as a quality metric is seemingly convincing.

In the context of the AHOPCA region, a CPR appeared very beneficial and in line with our goal of uniform regional pathology development. Hence, in this retrospective CPR of 156 specimens from 125 patients with a diagnosis of NB and treated under the same NB2012-AHOPCA guidelines, the original diagnosis of NB was confirmed in 88.5% of the specimens (138 of 156). However, an agreement between the original and revised histologic subclassification of the NB was noted in only 25.0% of the specimens (39 of 156). The most common reasons for this discrepancy were the lack of morphologic subclassification—33 specimens were initially signed out as NB but without further specification or subclassification (not otherwise specified)—when in fact they should have been classified as NB, Schwannian stroma poor, poorly differentiated (33 of 156, 21.2%). Another important reason for the discrepancy was the inappropriate subclassification of postchemotherapy NB specimens (21 specimens) when the posttherapy status precludes a histologic subclassification (21 of 156; 13.5%).

The age-associated histologic classification of NB combines the amount of Schwannian stroma content, degree of neuroblastic differentiation, and MKI, and in the end leads to the subcategorization in a favorable or unfavorable histopathology subgroup. It is apparent in this CPR that this subclassification is not consistently applied by the AHOPCA pathologists, even though it was implemented about 20 years ago.7 In fact, an agreement between the assigned MKI was observed in only 4 of 156 specimens (2.5%), and the initially given INPC and final CPR INPC were similar in only 18 specimens (18 of 156; 11.5%). In 127 specimens INPC was not specified in the original pathology report, but after CPR, 48 patients were categorized as having unfavorable histology and 28 patients classified with favorable histopathology. The other 51 cases remained without an assigned INPC. Indeed, it was not applicable in 42 samples, and it could not be determined in 9 specimens. The INPC category in NB is used to help determine the risk grouping of these patients and guide treatment selection. Therefore, not only is the exact diagnosis important, but providing adequate histologic subclassification and the INPC are also essential for the correct treatment of a patient with NB.

The second objective of this study was to implement manual MYCN IHC at HNNBB and evaluate its reliability compared with automated IHC and/or FISH assay to determine the MYCN status of patients with NB. The benchmark method to determine the MYCN status in patients with NB is FISH. We have previously demonstrated that automated IHC has a 91.66% sensitivity and 96.29% specificity compared with MYCN FISH in a pilot study of 78 patients.11 In this present study, manual MYCN IHC was performed in 120 specimens, with a definitive result in 89.2% (107 specimens), including 28 that showed bona fide evidence of MYCN protein expression (28 of 120; 23.3%) and 79 specimens (65.8%) with negative MYCN IHC. However, unlike when using the automated IHC stainer, there was a subset of the cases (10.8%) in which the interpretation of the IHC slide was equivocal—likely due to the background staining, which led to a questionable result (13 of 120 specimens). When repeated with an automated staining procedure, 11 of 13 questionable manual MYC IHC results showed negative IHC in 9 of them (9 of 13; 62%),

and 2 cases showed focal positivity (15.4%, 2 of 13). It is always our recommendation to reflex any questionable IHC result (manual or automated) to FISH testing.

Only a small number of cases in this cohort had FISH and/or automated IHC results previously done and available for comparison. However, the correlation between the 3 assays was very promising. For instance, all of the cases with the MYCN gene amplified by FISH exhibited evidence of protein expression by both automated and manual IHC. A single specimen classified as unfavorable histology that was previously reviewed and had MYCN gene not amplified, and negative automated IHC was interpreted as positive for protein expression by manual IHC. Another previously reviewed case, a post-therapy neuroblastoma specimen, showed questionable manual IHC results, but when stained by automated stainer, it was interpreted as positive for MYCN protein overexpression (FISH study was not previously performed in this case; Figure 3, B). The relatively low percentage of questionable results (10.8%) is very encouraging and can drastically decrease the number of cases that would require reflex to FISH and consequently would reduce the expenses in settings with limited resources. Moreover, centralizing the implementation of an MYCN IHC assay in a single center that could attend to the entire AHOPCA region seems to be not only an excellent cost-saving strategy but also important for capacity building and empowerment for this limited-resource region. In many low-income countries, such as those in the AHOPCA region, most of the pathologists are generalists and lack subspecialty training. The retrospective pathology review process itself provides an excellent opportunity for capacity building and the creation of regional expertise. For instance, the HNNBB had 39 patients with a diagnosis of NB between 2012 and 2017, which gives an average of 7.8 cases per year. During the 1 week of centralized review, the pathologist from HNNBB (A.P.) was exposed and able to jointly review 156 specimens, which would represent the number of NBs this pathologist would typically examine from HNNBB in 20 years. However, to ensure that a retrospective review can be a valuable setting for training, a prospective phase of diagnostic capability verification will need to take place.

A retrospective pathology review process involving multiple centers, in particular from different countries, is not exempt from its challenges, in particular in areas with limited resources. The cost of submitting samples by mail, as well as the time for clearing customs, needs to be carefully considered. The use of digital pathology may be a potential solution to minimize these issues,16,17 although the upfront investment necessary to acquire slide scanners, for example, would also need to be taken into consideration. A consistently high-quality H&E slide is also required before we contemplate the use of digital pathology. In that regard, unfortunately, 31.3% (25 of 80) of the original H&E-stained slides submitted for review still demonstrated poor or inadequate quality. Actions to consistently improve fixation, processing, the quality of the sectioning, and the quality of the staining in all anatomic pathology laboratories of the AHOPCA region have been our primordial goal. However, this study shows that we still have room for improvement. Another fundamental issue revealed by this retrospective pathology review was the lack of well-organized storage of pathology material in the AHOPCA laboratories, leading to missing paraffin blocks or unavailable pathology report(s).

Taken together, the results observed in this retrospective multicenter review of NB specimens highlight the need for the implementation of a CPR program to serve underserved regions. A CPR center would ensure that the correct pathology diagnosis is provided—including all the necessary subclassification to guide the treatment selection of patients with NB. Our next step is to initiate a prospective rapid review of all the NB cases from the AHOPCA region and to provide manual MYCN IHC to all confirmed NB cases, reflexing to FISH assay any questionable result; however, the use of a strict quality assurance program to validate the performance of the regional pathology referral center would be needed. This approach will further promote regional collaboration, improve test utilization, and ultimately impact the outcome of pediatric patients in this limited-resource region. A similar strategy of implementing a regional pathology referral center can be considered by other areas of the world where pathology expertise and resources are also limited.

The cost of performing a pathology review at a regional CPR center consists of consumables, reagents, personnel (eg, pathologists and technologists), and shipment fees. Whereas the initial operation of a CPR program can be achieved with the aid of external grants, the involvement of local government bodies and other regional sponsors is fundamental to the maintenance of such initiative in the long term. The main concept necessary to perpetuate this type of collaborative work is to make the key regional stakeholders comprehend that the cash invested in having an accurate diagnosis and correctly risk stratifying these patients will have the potential to impact patients' outcomes and, ultimately, represent savings by not spending money treating a patient based on a wrong diagnosis.

References

1. Luna-Fineman S, Barnoya M, Bonilla M, Fu L, Baez F, Rodriguez-Galindo C. Retinoblastoma in Central America: report from the Central American Association of Pediatric Hematology Oncology (AHOPCA). Pediatr Blood Cancer. 2012; 58(4):545–550.

2. Castellanos EM, Barrantes JC, Baez LF, et al. A chemotherapy only therapeutic approach to pediatric Hodgkin lymphoma: AHOPCA LH 1999. Pediatr Blood Cancer. 2014; 61(6):997–1002.

3. Navarrete M, Rossi E, Brivio E, et al. Treatment of childhood acute lymphoblastic leukemia in central America: a lower-middle income countries experience. Pediatr Blood Cancer. 2014; 61(5):803–809.

4. Ceppi F, Ortiz R, Antillon F, et al. Anaplastic large cell lymphoma in Central America: a report from the Central American Association of Pediatric Hematology Oncology (AHOPCA). Pediatr Blood Cancer. 2016; 63(1):78–82.

5. Santiago TC, Jenkins JJ. Histopathologic diagnosis of pediatric neoplasms: a review of international consultations. Arch Pathol Lab Med. 2013; 137(11):1648–1653.

6. Santiago T, Hayes C, Polanco AC, et al. Improving immunohistochemistry capability for pediatric cancer care in the Central American and Caribbean region: a report from the AHOPCA Pathology Working Group. J Glob Oncol. 2018; 4:1–9.

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

8. Cohn SL, Pearson AD, London WB, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. J Clinl Oncol. 2009; 27(2):289–297.

9. Shapiro DN, Valentine MB, Rowe ST, et al. Detection of N-myc gene amplification by fluorescence in situ hybridization: diagnostic utility for neuroblastoma. Am J Pathol. 1993; 142(5):1339–1346.

10. Quintana Y, Patel AN, Arreola M, Antillon FG, Ribeiro RC, Howard SC. POND4Kids: a global web-based database for pediatric hematology and oncology outcome evaluation and collaboration. Stud Health Technol Inform. 2013; 183:251–256.

11. Santiago T, Tarek N, Boulos F, et al. Correlation between MYCN gene status and MYCN protein expression in neuroblastoma: a pilot study to propose the use of MYCN immunohistochemistry in limited-resource areas. J Glob Oncol. 2019(5):1–7.

12. Vujanic GM, Sandstedt B, Kelsey A, Sebire NJ. Central pathology review in multi-center trials and studies: lessons from the nephroblastoma trials. Cancer. 2009; 115(9):1977–1983.

13. Jackson TJ, Williams RD, Brok J, et al. The diagnostic accuracy and clinical utility of pediatric renal tumor biopsy: report of the UK experience in the SIOP UK WT 2001 trial. Pediatr Blood Cancer. 2019; 66(6):e27627.

14. Vujani´c GM, Gessler M, Ooms AHAG, et al. The UMBRELLA SIOP–RTSG 2016 Wilms tumour pathology and molecular biology protocol. Nat Rev Urol. 2018; 15(11):693–701.

15. Teot LA, Sposto R, Khayat A, Qualman S, Reaman G, Parham D. The problems and promise of central pathology review: development of a standardized procedure for the Children's Oncology Group. Pediatr Dev Pathol. 2007; 10(3):199–207.

16. Mroz P, Parwani AV, Kulesza P. Central pathology review for phase III clinical trials: the enabling effect of virtual microscopy. Arch Pathol Lab Med. 2013; 137(4):492–495.

17. Hedvat CV. Digital microscopy: past, present, and future. Arch Pathol Lab Med. 2010; 134(11):1666–1670. Arch Pathol Lab Med. 2010; 134(11):1666–1670.