Principles and Practice of Surgical Pathology for the Diagnosis of Pediatric Head and Neck Diseases

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The most frequent tissue samples submitted to the Pediatric Surgical Pathology Laboratory for analyses and diagnosis are associated with three major categories of diseases: abnormal tissue growths or lesions that may or may not be neoplastic, inflamed or infectious disease tissues, and tissues removed from surgical correction of developmental anomalies or inflammatory conditions.

Light microscopy analysis of tissues (Histopathology) currently is the method of choice for routine diagnosis of surgical biopsies or excisions. For the diagnosis of complex diseases such as infections or neoplasms, microscopic evaluation is also an important initiating step to be followed with one or several adjunct analytical procedures (such as cytogenetic, flow cytometry, immunohistochemistry, and molecular testing) that may be necessary to establish an accurate diagnosis. To ensure an efficient time-line in making a final diagnosis for patient treatment and management, surgical pathology laboratories adopt standardized guidelines to implement the essential procedures to process the tissues submitted for examination, followed by the types of analysis according to the hospital environment (primary care centers versus academic health science centers) and the type(s) of information to be incorporated in the pathology report. Both principles and practice of these procedures are explained in this chapter.

Practical Guides for the Submission of Tissues to the Pathology Laboratory

An appropriate tissue preparation is crucial in rendering the particular type of diagnostic testing. This is important to know and to plan ahead of any procurement of disease tissues to be sent to the pathology laboratory. This is of particular importance regardless of whether the tissue is submitted to pathology laboratory as a biopsy with a diagnostic intent or after performing surgical excision with both a diagnostic and a curative intent.

Tissue biopsy for diagnosis of complex diseases, for example, neoplasms that require microscopic analysis followed by the appropriate adjunct diagnostic procedures, requires fresh tissues without fixation additives. For these samples, they must be submitted fresh. To prevent dehydration, the tissue wedges or cores should be wrapped gently with sterile physiological saline-soaked gauze or small amounts of sterile tissue culture fluids in an airtight container and delivered to the pathology laboratory. If the lesion requires microbiology or virology analyses, a separate sample should be obtained in the operation room under a sterile surgical field and submitted fresh to the microbiology/virology laboratory.

For surgical resections of the mass lesion, the surfaces of the tissue mass should be left intact. Appropriate marking sutures should be placed by the surgery team to denote the anatomical orientation of the tissue excised. In addition, if a particular region contains an important resection margin to be analyzed, this region should also be identified with a marking suture. If these tissues do not require complex analyses, formalin fixative can be added into the tissue container and then send to the pathology laboratory. If an intraoperative diagnosis is needed to guide surgical management with regards to the adequacy or completion of tumor resection, a fresh sample of the resection margin can be submitted to the pathology laboratory for a frozen section analysis.

For malformations (non-syndromic types), corrective surgeries for developmental anomalies, simple cysts, granulation tissues, nevi, tragus, and localized tissue growths associated with hyperplasia where malignancy is not clinically suspected or tissue debridement where microbiological cultures are not required, these resected tissues should be deposited in toto in formalin fixative, and send to the pathology laboratory for further analyses. For tissues that are submitted from patients with infectious agent, pathology laboratory should be informed of

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P. Campisi et al. (eds.), *Pediatric Head and Neck Textbook*, https://doi.org/10.1007/978-3-030-59265-3_3

their biohazard potentials. Appropriate labels should be attached to the specimen container.

For fine-needle aspirate samples, these should be fixed in cytolyte fixative (alcohol-based) and for aspirates of crystalline material, these aspirates should be fixed in alcohol (100% ethanol).

For skin biopsies where bullous diseases of autoimmune diseases are suspected, an additional fresh piece should be submitted to be frozen for immunofluorescence stains.

For samples that electron microscopy is needed, tissues should be fixed rapidly in glutaraldehyde/cacodylate buffer and stored cold (+4C). The pathology laboratory will further divide the tissue into small pieces.

Evaluation Procedures and Methods Used in the Pathology Laboratory

Tissue Examination

Upon arrival of surgical resection tissues/tissue masses in the pathology laboratory, the specimen must be weighed and recorded. The external dimensions must be taken and recorded. If a tissue mass is visible, dimensions of the lesion in these aspects: superior/inferior distance; anterior/posterior distance; and medial/lateral distance should be taken and recorded.

Photo-documentation: Surgical resections/excisions that involve an organ or part of an organ should be photographed always with a coded identifier and a measuring scale. The specimen should be laid out reflecting the anatomical position of the patient capturing the coronal or sagittal plane. Thereafter, the external surfaces of the tissue should be painted with special ink/dyes that are resistant to tissue processing fluids during the subsequent paraffin embedding and tissue sectioning procedures for microscopy. Ideally, multiple ink colors are used to mark the orientation of the tissue; for example, using different colors for the superior or inferior poles, the anterior/posterior surfaces, and the medial and lateral surfaces. The ink can be immobilized with exposure to a fixative of formal-acetic acid alcohol (a mixture of 25 ml formalin that contains 37% formaldehyde, 25 ml of glacial acetic acid, and 450 ml of alcohol).

Pending on the type of organ resected, and ideally, the specimen is cut open along the coronal, or sagittal or anterior/posterior plane or along the midline only, to allow the best visualization of the relationship of the lesion within the organ to its nearest resection margin for photo documentation. Note that for thyroidectomies where preservation of the capsule are required, they should not be cut open in the fresh state and opening the specimen by cutting should be delayed until after formalin fixation. Similarly, for enucleated tumor masses that have a thin veil surface covering, opening of the specimen may best be delayed until it has been hardened after formalin fixation.

For small biopsies other than core tissue biopsies, frequently such as those from the skin or a small single piece of nodular mass, marking the resection surface with surgical ink is mandatory.

For tumor lesions that are not visible until the specimen is cut open, dimensions of the lesion(s) similar to those visible externally must be taken. At least two photos should be taken (with calibrated scale placed adjacent to the lesion) where one of them will be used to provide a tissue sampling blocking template to aid in the completion of a documentation of the locations of where tissue samples have been taken later for paraffin block embedding for microscopy. In addition, the distance between the edge of the lesion to its nearest resection margin or any other surface margins must be taken and recorded.

For small tissue specimens such as oral odontogenic specimens that are submitted with cystic tissues and teeth, photo documentation is also important. After formalin fixation, all calcified tissues need to have the minerals removed (solutions containing dilutions of glacial acetic acid or EDTA) prior to sectioning.

General Guidelines for Tissue Sampling

For all practical purposes, all tissue requires fixation in buffered formalin (10% phosphate-buffered formalin contains a final concentration of 4% formaldehyde) for at least 24 h. For larger tissues, they will require longer fixation time. Large tissue sample may need to be sectioned serially into 1 cm thick slices to ensure adequate penetration of the formalin fixative. For the subsequent sampling of the tissue, 1 tissue block per 1 cm of tumor size that is representative of the abnormal gross appearance of the tissue is the routine recommendation. However, if the tumor exhibits unusual and unexpected appearances on gross examination, sampling in additional tissue blocks is recommended. The standard tissue cassettes size accommodates a maximum 3 × 2.5 × 0.4 cm piece of tissue sample.

Specific Guidelines for Tissue Sampling and Analytical Procedures for Neoplastic Surgical Specimens

Pediatric Head and Neck Neoplasms

Introduction

Pediatric Head and Neck malignancies unlike those in the adults are rare. A brief world-wide literature search was performed on the surveillance of Pediatric Head and Neck malignancy published after 2013. Qaisi M. and Eid I. from the United States in 2016 reported that pediatric H&N malignancies represent 12% of all pediatric malignancies and have an incidence of 1.49 cases per 1 million person-years [1]. They reported that the most common types are lymphoma (27%), neurotumors, including primitive neuroectoderm tumors (23%), thyroid malignancies (21%), and sarcomas (including rhabdomyosarcoma) (12%). Less common are nasopharyngeal carcinomas, skeletal/bone, and odontogenic malignancies, including osteosarcoma, Ewing, Langerhans histiocytosis, and ameloblastic carcinoma [1]. Another review published in 2018 from Brazil reported that pediatric H&N malignancies represent 5.1% of all of the 7181 pediatric cancers (registered between 1986 and 2016) and the mean age of diagnosis was 9.35 years [2]. Burkitt lymphoma (16.62%), nodular sclerosis Hodgkin lymphoma (13.08%), nasopharyngeal carcinoma (12.81%), and rhabdomyosarcoma (12.81%) were observed [2].

Head and Neck Epithelial Neoplasms

Carcinomas in children and adolescents in the head and neck region are rare. In the largest review of pediatric H&N non-nasopharyngeal squamous cell carcinomas, 159 patients we registered between 2004 and 2013 in the US National Cancer Database [3]. 6% of these patients were 0–4 years old, 9% were 5–10 years old, 22% were 11–15 years old, and 63% were 16–19 years old [3]. 55% involved the oral cavity, 14% in the larynx, 13% in the nasal cavity, 17% in the salivary gland, and 10% in the oropharynx. The most common stage group was Stage IV (33%). 72% were without HPV data status. 16% were unknown. 7% were registered as negative, 1% was positive for low-risk type, and 4% were of high-risk type. A National study from Denmark (1980–2014) reported 32 pediatric patients with H&N squamous cell carcinomas [4]. The most common location was in the oral cavity, accounting for 43%. None of their laryngeal carcinomas occurred in the age group between 0 and 17 years. All six cases of laryngeal carcinomas were 18–24 years old [4]. This review also pointed out that when pediatric H&N squamous cell carcinoma was observed, there was an association to predisposing factors such as DNA repair defects (Fanconi anemia, Bloom syndrome), prior irradiation of H&N among cancer survivors, oral graft versus host disease, or oncogenic viral infections (such as EBV in nasopharyngeal carcinomas). Sporadic case reports of rare pediatric H&N carcinomas in the current literature included a case of HPV+ve laryngeal carcinoma in a Fanconi anemia patient with additional NOTCH 1 mutation [5]; extremely rare sites such as the thyroglossal duct cyst [6, 7]; vocal cord [8]; lip [9]; branchial cleft cyst [10]; external auditory canal [11], and a large cell neuroendocrine carcinoma of the nasopharynx in a 9-year-old child [12]. Newly recognized highly aggressive malignant carcinoma such as NUT carcinoma of the facial midline [13–15] and mammary analog secretory carcinomas of the salivary gland [16] also emerged from the recent clinical and pathology literature.

As surgical resections are essential of the treatment for many of these conditions, even for their rarity, pediatric pathologist will encounter resections for examination and tissue evaluations, a discussion on specific examination protocols for cancer staging in this section is warranted. Lymph nodes for staging of H&N neoplasms are methodically examined and if they appear abnormal, they are excised and identified to the pathology laboratory according to the origin of their anatomical domain. The identifier comprises the uniform denotation terminology of Level I to VII, modified from the contents of Tables 5.1 and 5.2 AJCC [17]; also see Fig 3.1, from Fig 5.1, Chap. 5 AJCC [17]. Level I is further

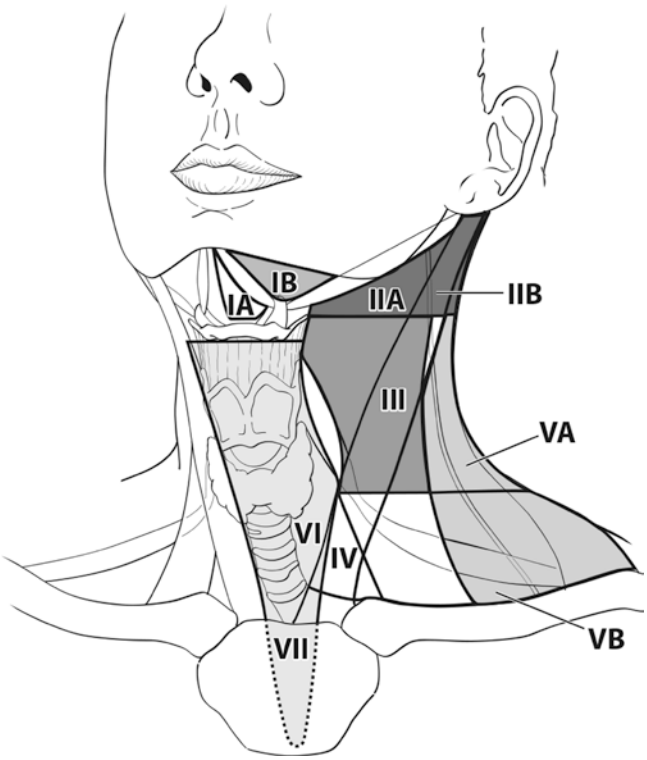

Fig. 3.1 Diagram indicating the location of lymph nodes in the neck. Used with permission of AJCC Cancer Staging Manual. 8th Edition, 2017. Amin MB. Editor in chief. Springer; Springer International Publishing, AG Switzerland

divided into Level Ia and Level I b. The anatomical boundaries of Level Ia are: Superior: Symphysis of the mandible; Inferior: Body of the hyoid; Anterior/medial: Anterior belly of the contra-lateral digastric muscle; Posterior/lateral: Anterior belly of the ipsilateral digastric muscle. Lymph nodes in this region are at risk of metastases from cancers arising from the floor of the mouth, anterior oral tongue, anterior mandibular alveolar ridge, and lower lip. For Level Ib, the anatomical boundaries are: Superior: Body of the mandible; Inferior: Posterior belly of the digastric muscle; Anterior/mandible: Anterior belly of the digastric muscle; Posterior/lateral: Stylohyoid muscle. The submandibular gland is usually removed with the lymph nodes within this triangle. Lymph nodes in this region are at risk of metastases from cancers arising from the oral cavity, anterior nasal cavity, skin and soft tissue structures of the midface, and the submandibular gland. Level II is also further subdivided into IIa and IIb. The anatomical boundaries of Level IIa are: Superior: Skull base; Inferior: Horizontal plane defined by the Inferior border of the hyoid bone; Anterior/medial: Stylohyoid muscle and Posterior: Vertical plane defined by the spinal accessory nerve. The anatomical boundaries of Level IIb are: Superior: Skull base; Inferior: Horizontal plane defined by the Inferior body of the hyoid bone; Anterior/medial: Vertical plane defined by the Spinal accessory nerve; and Posterior/lateral: Lateral border of the Sternocleidomastoid muscle. Lymph nodes in this region are at risk of metastases from cancers arising from the oral cavity, nasal cavity, nasopharynx, oropharynx, larynx, and parotid gland. The anatomical boundaries of Level III are: Superior: Horizontal plane defined by the inferior body of the hyoid; Inferior: Horizontal plane defined by the inferior border of the cricoid cartilage; Anterior/medial: Lateral border of the sternohyoid muscle; Posterior/lateral: Lateral body of the sternocleidomastoid or sensory branches of the cervical plexus. Lymph nodes in this region are at risk of metastases from cancers arising from the oral cavity, nasopharynx, oropharynx, hypopharynx, and larynx. The anatomical boundaries of Level IV are: Superior: Horizontal plane defined by the inferior border of the cricoid cartilage; Inferior: Clavicle; Anterior/medial: Lateral border of the sternohyoid muscle; Posterior/lateral: Lateral border of the sternocleidomastoid or sensory branches of the cervical plexus. Lymph nodes in this region are at risk of metastases from cancers arising from the hypopharynx, thyroid, cervical esophagus, and larynx. Level V is further divided into Va and Vb. The anatomical boundaries of Level Va are: Apex of the convergence of the sternocleidomastoid muscle and trapezius muscles; Inferior: Horizontal plane defined by the lower border of the cricoid cartilage; Anterior/medial: Posterior border of the sternocleidomastoid muscle or sensory branches of the cervical plexus; Posterior/lateral: Anterior border of the trapezius muscle. The anatomical boundaries of Level Vb are: Horizontal plane of the lower border of the cricoid cartilage; Inferior: Clavicle; Anterior/medial: Posterior border of the sternocleidomastoid muscle; Posterior/lateral: Anterior border of the trapezius muscle. Lymph nodes in this posterior triangle region are at risk of metastases from cancers arising from the nasopharynx, oropharynx, and cutaneous structures of the posterior scalp and neck. The anatomical boundaries of Level VI are: Superior: Hyoid bone; Inferior Suprasternal notch; Anterior/medial: Common carotid; Posterior/lateral: Common Carotid artery. Lymph nodes in this region are at risk of metastases from cancers arising from the thyroid glands, glottis and subglottic larynx, apex of the piriform sinus, and cervical esophagus. The anatomical boundaries of Level VII are: Superior: Suprasternal notch; Inferior: Innominate artery; Anterior/medial: Sternum; Posterior/lateral: Trachea, esophagus, and prevertebral fascia. Lymph nodes in this region are at risk of metastases from cancers arising from the thyroid and the esophagus.

In addition to the Lymph node groups described above, other lymph node sites pertinent to assessment in H&N neoplasm include sub-occipital, retropharyngeal, parapharyngeal, buccinators (facial), pre-auricular and peri-parotid and intra-parotid.

Examination of the resected tissues and lymph nodes is essential to capture the pertinent data that are needed to be entered into the final pathology report to fulfill the TMN categorization of these tumors according to the recommendation of the ACC [17]. The histopathology information is useful to guide post-operative treatment and prognosis of the tumor.

Pediatric Sarcoma

Unique to most of the malignant pediatric sarcomas, the neoplastic cells are primitive and display very little light microscopic features indicative of their cell of origin. Under the routine hematoxylin and eosin (H&E) stain of the tissue section, the neoplastic cells characteristically show small hyperchromatic (blue-stained) nuclei with scant amounts of pale eosinophilic cytoplasm. The term "small round blue cell tumors" are frequently used to describe them. Other revealing histopathology features may be the frequency of mitosis, apoptosis, and the abundance of nuclear anaplasia (defined by the presence of tumor cells that have nucleus 4× larger than the adjacent tumor cells with or without abnormal large atypical mitotic figures). Final diagnosis requires utilization of a comprehensive set of adjunct analysis that includes histochemistry, immunohistochemistry, cytogenetics, and molecular analyses [18]. For cytogenetic analyses and complex molecular procedures, submission of fresh tumor tissues to the pathology laboratory is required. Use of frozen section analysis currently plays a very minor role in providing a diagnosis.

Pediatric Lymphoma and Lymphoproliferative Diseases

Microscopic examination of sections of well-fixed lymphoid tissue is an important initial step toward a diagnosis. Similar to pediatric sarcoma, final diagnosis requires extensive analysis with adjunct analytical procedures. They include immunohistochemistry, flow-cytometry, cytogenetics, and molecular analysis [19]. As a result, all lymphoma samples require fresh tissue submitted to the pathology laboratory for adjunct analysis. Use of frozen section analysis currently plays a very minor role in providing a diagnosis.

Adjunct Diagnostic Procedures

Analysis for Abnormal Tissue Components or Infectious Agents with Histochemistry

Histochemistry stains are useful to detect the presence of abnormal tissues matrix (e.g., sulfated proteoglycans; mucin; elastin, collagen, basement membrane proteins or amyloid) deposits of unusual substances extrinsic to the natural environment of normal tissue (e.g., iron, or copper), or unusual accumulation of granules, metabolites within cytoplasm of the abnormal cells (e.g., melanin, glycogen, lysosomes, myeloperoxidase, argentaffin, and argyrophil granules (exclusively for endocrine cells); or cytoplasmic mucin). The menu of histochemistry stains available for the specific detection of these substances is as follows:

The most frequent usages are hematoxylin and eosin for all tissue slides; Elastic trichrome to demonstrate elastin fibers and collagen; Movat pentachrome stain for vascular tissues or Snooks reticulin, they can demonstrate elastin fibers and the Movat pentachrome stain also identifies collagen in yellow and cells in red; Periodic acid-Schiff (PAS) and PAS with diastase treatment—PASD to distinguish glycoprotein from glycogen; Oil red O to demonstrate fat/cytoplasmic lipid; Mucicarmine or Alcian Blue(for acid mucin) or Hale stain to demonstrate mucin or proteoglycans (by the latter); and Fontanna-Masson for melanin. Other less common stains are: iron stain, copper stain, Congo red for amyloid, Von Kossa calcium stain; Phospho-tungstic acid hematoxylin (Mallory PTAH) for neuro lesions and muscle differentiation. For hematological cells and tissues, Giemsa for lymphoid/myeloid cell cytology and for granules in mast cells. Toluidine blue for mast cells, Chloroacetate for myeloid leukemia cells in tissues; Wright stain for blood smears.

Similarly, a limited numbers of histochemistry are also available to detect microbial pathogens in tissues.

These stains include gram stain for bacteria; Grocott-methenamine-silver nitrate (GMS) stain for fungi, Ziehl-Neelson (ZN) stain for mycobacteria Warthin Starry stain for Spirocheles or Bartonelle species, Steiner stain for bacteria and spirochetes, Dieterle stain for Spirochetes, and mucicarmine to detect Cryptococcus. Further identification, subtyping, and identifying the antibiotic sensitivities of these pathogens are best analyzed by microbiologist via the further application of culture and or molecular procedures (e.g., polymerase chain reaction (PCR)-mediated DNA amplification techniques). For histological samples, Epstein–Barr virus (EBV) can be detected by in-situ hybridization of the EBV-encoded RNA. Similarly, in-situ hybridization procedures are applicable to HPV subtyping. Immunostains are available for the detection of cytomegalovirus, adenovirus, herpes virus, or HHV6.

Immunohistochemistry

As most of the antibodies from commercial sources now are produced to formalin-treated antigen epitopes, immunohistochemistry stains today can be done on formalin-fixed paraffin-embedded tissue sections. Application of immunohistological stain is most useful to analyze tumor. For most of the diagnoses made for non-neoplastic tissues, with the exception of lymphoproliferative diseases or some dermopathies, immunohistochemistry may not provide additional diagnostic advantages. When applied to pediatric small round blue cell tumors, it can provide evidence of the histogenic origin of the neoplastic tissue and together with the knowledge of some of their association with unique expression of certain proteins, these primitive neoplasms can be subclassified with accuracy. Based on my experience, with few exceptions, only a few tumors have association with a unique/specific protein expression (e.g., NUT midline carcinomas with expression of nuclear protein of testes) [14]. In a majority of tumor screening analyses, a customized panel of multiple antibodies is required. Listed below are examples of several useful customized panels of antibodies that are useful to screen pediatric tumors in order to group them into a particular tissue tumor domain and to narrow the pathological differential diagnosis. Specific immunopathology details that best define each individual type of pediatric tumor are discussed in their respective chapters.

Useful Antibody Panels Customized to Screen Pediatric Soft Tissue Neoplasm and Some Cutaneous Lesions

Use of CD99, myogenin, desmin, smooth muscle actin, actin, vimentin, S100, pan-cytokeratin, epithelial membrane antigen, and CD45 in this panel would be a reasonable first start. Pending on the results, a second panel comprising a few selected from some of these antibodies will further narrow the differential diagnosis within the soft tissue tumor, or pediatric small round blue cell tumors: beta-catenin, TLE-1, Bcl2, CD56, CD57, neuron-specific enolase, synaptophysin, neurofilament, CD117, SOX 10 and ALK-1; for malignant bone lesions, SATB2 or CD207, S100 and CD1a for Langerhans cell histiocytosis; for cutaneous lesions, Factor

XIIIa CD34, CD68, CD163, S100, CD207, SOX 10, Mel A, or HMB45. For vascular lesions, smooth muscle actin, Factor VIII, CD31, CD34, D2-40, and Glut-1 are helpful. For rarer pediatric sarcoma, the additional third panel of antibodies that comprise BAF47, CCNB3, MiTF, or TFE3 may further define a rare/smaller subset of sarcomas such as undifferentiated sarcoma, Ewing sarcoma-like tumors or poorly differentiated synovial sarcoma with *BCOR–CCNB3* fusion or alveolar soft part sarcoma respectively, as examples. For further details of immunophenotypes of each type of sarcoma, please see the chapter on sarcoma.

Useful Antibody Panel Customized to Screen Lymphoid Neoplasm

Lymphoma comprises two distinct entities: Hodgkin and non-Hodgkin lymphoma. Both have distinct immunopathological characteristics. Hodgkin lymphoma is associated with the presence of Reed Sternberg cells and a subtype of Hodgkin lymphoma: Nodular lymphocyte predominant Hodgkin lymphoma has atypical lymphohistiocytic cells. The immunostain expression of Reed Sternberg cells are: CD15+, CD30+, CD45-ve, PAX 5 weak +; CD20-ve in a majority of cases; ALK1-ve and T-cell antigen negative in a vast majority of cases. The immunostain panel results for nodular lymphocyte predominant Hodgkin lymphoma are CD30+, CD20+, CD45+, PAX 5 weak +; CD15+/− and T-cell antigen negative. A collarette of PD1+ or CD57+ small lymphocytes surrounding the atypical lymphohistiocytic cells can be present [19].

The nomenclature of Hodgkin lymphoma is currently designated according to the origin of lymphocyte subsets within various domains of the normal lymph node. A large number of lymphocyte markers are needed for the identification of neoplastic cells. Not infrequently, lymph nodes are abnormal in size due to the presence of leukemia cells rather than lymphoma. Application of immunohistochemistry using myeloperoxidase immunostain can be useful to identify the presence of myeloid malignancy. As lymphomas can arise from immature or mature B cells or T cells, the use of TdT immunostains can identify immature lymphomas which show positive nuclear staining.

For the initial screening of non-Hodgkin lymphoma (in the absence of leukemia), a panel of B-cell and T-cell-specific antibodies that include CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD15, CD20, CD30, CD43, CD45, CD45RO, CD79a, TdT, and ALK1 should be adequate. Detailed association of additional markers with specific lymphoma subtypes can be found in the lymphoma chapter. For the assessment of post-transplant lymphoproliferative disease, detection of EBV by in situ hybridization should be included. For assessment of primary immunodeficiencies, immunohistological assessment of the thymus can be helpful, a panel of antibodies that includes CD1a, CD2, CD3, CD4, CD5, CD7, CD8, S100, T-reg (i.e., FOX P3), S100, CD68, pan-cytokeratin, and CK8 are important. A portion of fresh thymic tissues should be sent to clinical immunology lab for in vitro lymphocyte functional testing and further testing for the expression of other T-cell functional antigens by flow cytometry.

Flow cytometric analysis is performed from cell suspension of the mononuclear cells population retrieved from teasing the fresh lymph node in vitro in the laboratory. The flow cytometer is equipped with cell surface staining with a number of antibodies tagged by fluorescent markers. The staining results are quantitated via a detector. It is rapid and for pediatric hematological neoplasms, it is applicable to detection of leukemia, and B-cell lymphomas. However, for the analyses of Hodgkin lymphoma and T-cell lymphomas, the use of immunohistopathology is more reliable.

Useful Antibody Panel Customized to Screen Pediatric Epithelial Neoplasm

Pan-cytokeratin, CAM 5.2, vimentin, epithelial membrane antigen, carcinoembryonic antigen (CEA), S100, actin, desmin, and smooth muscle actin are initially applied. Then according to the histopathological differential diagnosis, additional antibodies are used to guide in determination of the final diagnosis, as discussed in the following examples.

For thyroid tumors inclusion of TTF1, CK5/6, CK7, CK20, 34betaE12, and p63 are useful. Calcitonin to be added for medullary thyroid carcinoma and CK19, calcitonin, p27, chromogranin, synaptophysin, Rb protein for parathyroid tumors.

For some salivary gland tumors, addition of CK5/6, CK7, CK20, 34betaE12, and p63 are important. For mammary analogue carcinoma of salivary glands, inclusion of mammaglobin with cytokeratin is important.

For immunostains of mediastinal germ cell tumors, a combination of pan-cytokeratin, Sox2, Oct-4, alpha fetoprotein, PLAP, Beta-HCG, and Sall4 will be useful.

The results from this antibody panel are not entirely foolproof. For example, sarcoma known as epithelioid sarcoma will express some of these markers. This highlights the importance of not relying completely on the application of immune-histopathology alone for diagnosis. Any final diagnosis cannot be made without the context of pathological information from histopathology as well as other testing results such as cytogenetic and or molecular procedures described in the following paragraphs.

Recently, immunostain of some types of pediatric tumors offers an additional role in determining the eligibility of patients to enroll in phase 2 or phase 3 clinical trial of targeted therapy with bio-active small synthetic molecule agents or immune checkpoint blockade modifiers at major academic health science centers. One example in Pediatric

Pathology is the frequent request to perform BRAF-v600 immunostain of thyroid carcinoma, systemic Langerhans cell histiocytosis (infrequently, for Erdheim-Chester disease, due to its rarity in children and adolescents), and melanoma. Positive immunostain of this BRAF-V600 corresponds to the presence of *BRAF* gene mutation. In thyroid carcinomas presence of this mutation has been correlated with more aggressive and iodine-resistant phenotypes [20]. In the case studies of Langerhans cell histiocytosis, *BRAF* activating mutation (50% prevalence) or in frame deletion of its exon 12 (6% prevalence) or somatic *MAP 2K1* mutation 20% prevalence) results showed all exhibited a central role in ERK phosphorylation/activation of the MAP-Kinase pathway and currently, clinical response has been reported by the administration of pharmacologically active ERK1/2phosphorylation inhibitors such as vemurafenib, dabrafenib or upon failing this, with PLX8394 and trametinib [21]. Similar example is the use of vemurafenib and cobimetinib for the treatment of metastatic melanoma [22]. Another example of new therapeutic approaches is to target the blockade of the PD1-PDL1 for Hodgkin and non-Hodgkin B-cell lymphoma [23]. In such therapeutic trials treatment with the addition of PD1 inhibitors (monoclonal antibody to PD1 ligand Nivolumab) were evaluated [24]. Immunostain of lymphoma cells using PDL1 plays a vital role in patient selection for these trials.

Cytogenetic Analyses

Cytogenetic abnormalities have been observed in a limited number of pediatric conditions. These range from abnormalities associated with some syndromic conditions, to some benign tumors as well as several malignant neoplasms. Examples of abnormal genetic findings found in syndromic conditions are germline mutations in *RET*, *MEN2A*, and *MEN2B* associated with multiple tumors manifesting as medullary carcinoma of the thyroid, or paraganglioma; germline *P53* mutations in patients with Li-Fraumeni syndrome that develop soft tissue tumors. *NF2* germline mutations in neurofibromas, vestibular schwannoma; alterations/inactivating mutations in the *PTCH1* gene in the odontogenic keratocysts of both syndromic (Gorlin–Goltz/nevoid basal cell carcinoma syndrome) [25, 26] and sporadic odontogenic keratocysts as well as sporadic developmental cysts including dentigerous cysts [27, 28].

Some cytogenetic abnormalities are seen in benign lesions such as dentigerous cysts that exhibit loss of heterozygosity in the *PTCH1* gene, USP6 fusions in 50% cases of aneurysmal bone cysts [29], and similar genetic findings in some cases of nodular fasciitis. *PLAG* gene alteration in lipoblastoma as well as in smaller number of cases of lipomas [30].

In some conditions, the same abnormality can be associated with different diseases. Such examples are the *ETV6–NTRK3* fusion in infantile fibrosarcoma [31], mammary analogue carcinoma of the salivary gland [32] or the presence of USP6 fusions in bone cyst or nodular fasciitis [29]. This non-specificity feature highlights the importance of the necessity of integrating the cytogenetic findings together with other diagnostic parameters.

In the analysis of malignant neoplasms, the most benefits of the cytogenetic information are the important role it has in the refinement of diagnosis of pediatric soft tissue tumors and lymphomas, especially in the diagnosis of small round blue cell tumors. In addition, for pediatric rhabdomyosarcoma, not only does the presence of *PAX3* or *PAX5* gene translocation to *FOXO-1*(*FKHR*) associated with t(2;13)(q35;q14) or t(1;13)(p36;q14) respectively defines the diagnosis of alveolar rhabdomyosarcoma, the type of fusion gene partner is specifically associated with the prognosis within this tumor (i.e., *PAX3–FKHR* fusion is unfavorable with marked inferior outcome than tumors with *PAX5–FKHR* fusion). Many other gene fusion anomalies which have important diagnostic values have been associated with various types of sarcomas and lymphomas. These are described in the corresponding chapters.

A few cytogenetic abnormalities have also been found in other non-soft tissue tumors. For example: t(3;8)(p21;q12); *CTNNB1–PLAG1* fusion in >50% pleomorphic adenoma the t(8q12); *PLAG1* fusion in <20 % pleomorphic adenomas; the t(11;19)(q21;p13); *MECT1–MAML2* fusion in mucoepidermoid carcinoma [33]. The t(12;15)(p13;q25)*ETV6–NTRK3* fusion in mammary analogue carcinoma of the salivary gland [32]. 8q12 rearrangement involving the PLAG oncogene in >80% lipoblastoma [30]. Other examples of cytogenetic anomalies are described in the corresponding chapter.

Analyses with Molecular Procedures

Molecular diagnosis can be performed with several technological platforms and I would like to group them and discuss their application to surgical pathology samples in two broad categories. The first category are the molecular tests that are designed to detect specific DNA alterations or sequence abnormalities that can be completed with a reasonable turnaround time in frame with the expected time taken to issue a surgical pathology report for diagnosis and management.

The second category are the molecular genome analyses with analytical platforms that generate a huge volume of data with an overwhelming complexity that require lengthy analyses by bioinformaticians for proper interpretations. The level of equipment and laboratory manpower support has limited the application of these analyses to major academic health science centers. These analyses are performed on specimens from patients who consent to participate and enroll in clinical treatment trials for the evaluation of the efficacy of personalized or targeted cancer therapy. Some of the molecular analyses may discover previously unknown disease mechanisms that could a specific hallmark feature of

that disease. Such information could be proven to be essential in the diagnosis of that disease entity. As future technology improvements in the speed of analyses of big data via the additional implementation of artificial intelligence for interpretation, and when the cost of these analyses becomes lower, and together with positive evidences of benefits from these clinical treatment trials, it is our hope that the molecular tests within this category will become available as part of an adjunct analysis to enrich the information within the diagnostic reports.

Molecular Tests that Have Positive Value to the Disease Diagnosis

Gene amplification procedures (polymerase chain reaction, PCR) are rapid analytical procedures that can be designed to analyze specific single gene locus such as the antigen receptor gene of lymphocytes to determine the presence or absence of clonal lymphoproliferations [34]. It can also be designed to detect known abnormal gene fusion abnormalities by using primers that have DNA sequences that are homologous to the flanking regions around specific gene fusion breakpoints provided the fusion regions are tightly clustered [35]. This single gene detection procedure broadens the detection to more than one type of gene fusions by expanding the primer repertoire that recognizes and binds to flanking regions of different types of gene fusion known to specific types of tumors. This multiple primer panel design permits to scale down the number of procedures needed for rapid diagnosis. Recently, other ultra-sensitive rapid screenings for nucleic acid abnormalities in tumors are available by the use of gene hybridization platforms by directly capturing, imaging, and counting fluorescent barcoded DNA primers, designed to recognize and hybridize multiple sequences present in known genes present in specific abnormal DNA molecules specific to each of numerous types of tumors incorporated in the reaction mixture, as much as 800 gene targets can be screen in each run of the assay (e.g., NanoString procedures) [36]. This is done without amplification or reverse transcription and circumvent the deleterious effects on this assay due to nucleic acid degradation. This assay is capable of sensitive and precise quantification of mRNA, miRNA, or DNA (for copy number variation or ChIP-Seq screening) as well as the characterization of gene fusions, splice variants [37, 38].

Challenges common to all of these nucleic acid detection platforms lie in the integrity of the source of DNA or RNA from the lesional tissues (fresh, frozen, or post formalin-fixed paraffin-embedded tissues). In general, gene hybridization platforms are less vulnerable to nucleic acid degradation but are less sensitive and gene amplification platforms are more prone to the effects of poor nucleic acid quality but are more sensitive in detection.

Polymerase Chain Reaction (PCR), for Qualitative Assessment

These DNA tests are based on using DNA (or reverse transcriptase generated c-DNA) amplification procedures known as polymerase chain reaction (PCR) to detect the presence of abnormal gene fusions (in specific types of soft tissue neoplasms). The design of this type of DNA analysis is based on the known structural gene abnormalities observed by cytogenetic analyses, followed by the subsequent cloning studies to obtain the specific DNA sequence at the gene fusion junction and thus the finding is complementary to the cytogenetic results. The sensitivity of this PCR analysis is however remarkably enhanced. It can detect an abnormal DNA fusion motiff within a tumor cell within one million normal cells. This test is applicable to tumor cell diagnosis as well as the assessment of minimum residual disease. This assay technique has also been expanded to screen for multiple tumor types by the introduction of various sets of oligonucleotide primers (amplimers) with specificities to each type of gene fusions (multiplex PCR). This procedure has also been applied to detect the presence of clonal lymphocytes (a hallmark pathological feature of lymphoma cells) by designing amplification DNA primers that target and hybridize to the known invariable DNA sequences that flank the variable regions of antigen receptor genes of lymphocytes.

qPCR for Relative Quantitation

By using the same cyclical DNA amplification procedure principle in the presence of thermostable DNA polymerase with an addition of a synthetic reporter probe which is tagged by mutually quenching fluors at each end that can hybridize to the specific abnormal DNA sequence to the DNA amplification probe mixture, and as the DNA synthesis progresses to completion during this procedure, one of the quenching fluor will be released and the newly synthesized DNA products become detectable and measurable in a real-time PCR machine. The geometric increase which corresponds to an exponential increase of the product can be used to determine the quantification cycle in each reaction. By performing the same amplification kinetics using primers specific to normal house-keeping genes, one can compare the relative quantity of normal versus the abnormal DNA templates or in the case of analyzing the relative differences in the expressed genes between normal and abnormal samples by using c-DNA as the initiating templates for the PCR.

Droplet Digital PCR for Absolute Quantification

Target-specific amplification of nucleic acid for detection remains the design backbone of this analytical platform. The main distinction is that the reaction mixture is separated into thousands to millions of partitions which is followed by a real-time or an endpoint detection of the amplified products.

The distribution of target sequences into these partitions typically would follow the Poisson distribution. This would allow an accurate and absolute quantification of the target from the ratio of positive against all partitions at the end of the amplification reaction. This procedure has the added advantage that reference materials with known target concentrations are not needed and it also has increased accuracy of quantification at low target concentrations compared to qPCR. This procedure is also more resilient to the presence of inhibitors in different types of samples [39]. Applications of this procedure to detect copy number variations in genes have been described [40].

Molecular Tests That are Valuable to Clinical Research and are Beneficial to Support Clinical Trials of Targeted Therapies

Currently in the field of medical oncology, developing more specific and less damaging therapies especially for children with malignancies based on the identification of tumor-associated so-called "driver genes" to be targeted with custom-made agents has become the central rationale of conducting gene targeting clinical trials. Next-generation sequencing (NGS) procedures have demonstrated to have the ability to identify specific gene alterations as key oncogenic drivers as novel actionable drug target(s) from tumor nucleic acids. The results of these types of analyses may be used to guide clinical treatment decisions in clinical trials. Advances in bioengineering toward automated DNA sequencing led to the development of rapid massive parallel DNA sequencing platforms for human genes. Several major commercially available platforms, such as the fluorescent imaging-based Illumina NGS system and the Pacific Biosciences Single Molecule real Time (SMRT) NGS platform; Ion Torrent and the Nanopore technology (Oxford Nanopore and Genia [Roche]) are available. The details of the sequencing technology behind them will not be described here, please see reference [41]. NGS procedures used to support these oncology trials are modified not to engage in seeking casual variants (which is extremely time-consuming) but to screen for the presence of single nucleotide variants, short insertions and deletions (indels), copy number variations (CNVs), and other structural variants of a panel of known tumor oncogenes and tumor suppressor genes. Hence this procedure is known as targeted NGS. The huge volume of DNA sequences obtained have to be subjected to bioinformatics tools (no less than 26 currently available) to conduct analyses and the quality of data is critically dependent on the type of algorithms engaged to create consensus and accuracy in the identification of true Cancer driver Genes [42].

Recent comprehensive genome analyses of 961 tumors from childhood cancers discovered 24 distinct molecular types of cancer. There were 149 putative cancer driver genes that separate these tumors into two classes: small mutation and structural/copy number variant (correlating with germline variants). Structural variants, hyperdiploidy, and chromothripsis are linked to TP53 mutation status and mutational signatures. An unambiguous predisposing germline variant was observed in 7–8% of the children in this study and 50% of the neoplasms harbor a potentially druggable event. These results are relevant for the design of clinical trials in the future [43].

The time required for targeted NGS analysis of neoplastic tissues is costly and lengthy and exceeds the turnaround time for the practice of surgical pathology. By scaling the types of targeted genes to a specific type of malignancy and selecting only genes that are prevalent in certain types of malignancies to be targeted (e.g., pediatric sarcomas and or myeloid malignancies) as seen in one example of NGS using OncoKids (a custom-designed NGS platform) or TruSight sequencing panels from Illumina, key driver mutations (such as *SMARCB1* in Rhabdoid tumors, *Alk* point mutations in neuroblastomas), or gene fusions results can be obtained for purpose of diagnostic, prognostic, and therapeutic applications [44].

Formulation of the Diagnostic Pathology Report

The Basic Essentials to Enter into a Pathology Report in a Free Text Format

Surgical pathology reports for non-neoplastic tissues are structured in a free text format that preferably include gross specimen information, microscopic description pertinent to the applicable diagnostic criteria, results of other studies, for example, microbiological stain results, connective tissue stain results or other histochemistry stain results, immunostain results, electron microscopy results, and/or genetic/cytogenetic results if applicable along with the final diagnosis.

Free text surgical pathology reports for neoplasia diagnosis should include the surgical procedure, the site of origin classification of the tumor, tumor size (e.g., maximum diameter), and the status of the tumor resection margin (gross measurements or microscopic status) (as in appropriate situations). In the microscopic description, pertinent cytological features, the degree of differentiation, that is grade (with the exception of lymphomas), presence or absence of necrosis and apoptosis, the presence of absence of metastasis, vascular, neuroinvasion, and status of tumor capsule with regard to the tumor growth should be reported. The number of lymph nodes, and the number of lymph nodes with tumor metastasis and the pathological stage should be examined. In the microscopic description, for sarcomas, tumor grade and mitoses should be included [45]. For melanomas, the depth of lesion from the epidermis is important to report. Results of other studies, for example, connective tissue stain results or

other histochemistry stain results, immunostain results, electron microscopy results and/or genetic/cytogenetic results if applicable should be included along with the final diagnosis.

The Essentials of a Specialized Pathology Report for Pediatric H&N Resection for Neoplasms

Over the past decades, cancer staging has been shown to play a central role in the improvement of cancer care. It has provided benchmarks and standards to define cancer prognosis and for the determination of best treatment for the specific type of cancer. Since 1997, The American Joint Committee on Cancer (AJCC) published a manual for cancer staging. This manual has since been periodically updated. The summary of part of the guideline for capturing the pertinent pathology aspects of cancers at specific H&N sites listed for the fulfillment of pathologist's role in cancer staging is based on the current 8th edition of AJCC Cancer Staging Manual published in 2017 [16]. Moreover, pathology reports for childhood neoplasms have a role to support ongoing multicenter international oncology trials in providing essential data that are important for the clinical and pathological evaluation of these trials with respect to the evaluation of pathology-related prognostic factors [45].

The Synoptic Pathology Report for Pediatric Head and Neck Neoplasms

Based on the original and primary source of information in the AJCC Cancer Staging Manual, Eighth Edition (2016) published by Spinger, Science+Business Media, The College of American Pathologists (CAP) posted revised Cancer Protocols (February 27, 2019) that are accessible from their website at www.cap.org/cancerprotocols. The CAP authorizes use of these protocols by physicians and other health care providers in reporting on surgical specimens, in teaching, and in carrying out medical research for non-profit purposes. Protocols for that are pertinent to this book that include: Thyroid carcinoma, thyroid biomarker reporting; head and neck protocols that include the larynx, lip, and oral cavity, major salivary glands, nasal cavity and paranasal sinuses, pharynx, squamous cell carcinomas, head and neck biomarker reporting; hematologic neoplasms protocols that include bone marrow, Hodgkin lymphoma, non-Hodgkin lymphoma, ocular adnexal lymphoma; pediatric neoplasm protocols that include Ewing, germ cell tumor, neuroblastoma, rhabdomyosarcoma; skin protocols that include melanoma; and others such as soft tissue tumor and the thymus. With the initiative of introducing these protocols, free text reporting of neoplasms such as the ones described in the previous section would eventually be replaced as free text reporting usually lack diagnostic parameters and hence is regarded as incomplete. CAP permits the use of these protocols for the purpose of issuing pathology reports for cancer diagnosis.

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