Cytopathology of Pediatric Malignancies

Where Are We Today With Fine-Needle Aspiration Biopsies in Pediatric Oncology?

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Pediatric malignancies are uncommon and many have overlapping morphologic features, which together present diagnostic challenges. Cytopathology is recognized as an accurate and cost-effective modality for the diagnosis of pediatric malignancies in resource-limited countries, but is underused for this purpose in the United States. This review focuses on the cytopathology of pediatric malignancies with the goal of demystifying cytologic diagnoses of these entities. Differences between malignancies in young patients and adults are discussed, and key epidemiological features of childhood malignancies are highlighted. In addition, the use of cytopathology in different geographical settings is contrasted to illustrate the impact of variable usage on the incidence of malignancy and the types of tumors observed in cytologic specimens. A review of the pattern-based approach to differential diagnosis is also incorporated, including the cytomorphologic features and ancillary studies that help to distinguish between various malignancies within each pattern. ***Cancer (Cancer Cytopathol)* 2014;122:322-36.** *© 2014 American Cancer Society.*

KEY WORDS: children; cytopathology; fine-needle aspiration; pediatric; malignancy; tumor; young.

INTRODUCTION

Pediatric malignancies often present diagnostic challenges, particularly in small biopsies and cytologic specimens. This is partly attributed to the overall rarity of these tumors, the different risk factors and associations, and the different spectrum of entities in comparison with tumors arising in adults, and is compounded by morphologic similarities between various tumors.[1] This challenge is accentuated on a global level, because 86% of the world's pediatric population lives in developing countries and this percentage is expected to rise over time.[2] In developing countries, pediatric malignancies are disproportionately represented and comprise up to 2% of all cancers, in contrast to Europe and the United States in which they represent approximately 0.5% of all cancers.[2] Furthermore, although infectious diseases are far more prevalent than cancer in developing countries, the mortality rate associated with cancer is greater, largely due to delayed diagnosis, comorbidities, and a lack of access to modern treatments in resource-limited settings. Fine-needle aspiration biopsy (FNAB) is routinely used for the evaluation of suspected pediatric malignancies in resource-limited settings and has proven to be an accurate diagnostic tool.[3,4] In contrast, physicians in the United States have been slow to embrace this modality, despite its being less costly and less invasive than either core needle or open biopsy. The objective of the current review is to provide a framework for approaching the cytologic diagnosis of pediatric malignancies and to raise awareness and acceptance of the value of this modality in resource-rich settings.

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Childhood malignancies are infrequent and differ from adult malignancies clinically and pathologically. This review discusses childhood malignancies, focusing on their frequency in different age groups, representation in cytologic specimens in different geographic regions, the use of cytopathology as a diagnostic tool in the United States, and cytological features of selected tumors using a pattern-based approach.

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TABLE 1. The 12 Main Diagnostic Groups in the International Classification of Childhood Cancer, Third Edition[1]

I. Leukemias, myeloproliferative diseases, and myelodysplastic diseases
II. Lymphomas and reticuloendothelial neoplasms
III. Central nervous system and miscellaneous intracranial and intraspinal neoplasms
IV. Neuroblastoma and other peripheral nervous system tumors
V. Retinoblastomas
VI. Renal tumors
VII. Hepatic tumors
VIII. Malignant bone tumors
IX. Soft tissue and extraosseous sarcomas
X. Germ cell tumors, trophoblastic tumors, and neoplasms of gonads
XI. Other malignant epithelial tumors and malignant melanoma
XII. Other and not otherwise specified malignant neoplasms

CHILDHOOD MALIGNANCIES: TUMOR TYPES, FREQUENCY, AND CORRELATION WITH AGE

The incidence of malignant neoplasms in children and adolescents is difficult to estimate in individual regions due to the rarity of these tumors, and thus population-based cancer registries provide unique and important data. The Automated Childhood Cancer Information System (ACCIS) project represents > 60 registries in 19 European countries and uses the International Classification of Childhood Cancer (ICCC), which is designed to allow robust comparisons across geographic regions and time. The third edition of the ICCC (ICCC-3) classifies tumors in 12 main groups (Table 1).[1] Based on the ACCIS data, children and adolescents aged < 20 years comprise ≤ 1% of all patients with cancers; however, the incidence rate has steadily increased over the past 25 years.[1,5,6] The increase involves most groups of tumors, with the exception of bone tumors, hepatic tumors, and retinoblastoma, in which little or no change in overall incidence was observed.[6] This increase is partly attributable to better diagnostic capabilities and more precise characterization of tumors, as evidenced by a decrease in unclassifiable lesions over time. However, the increase may also reflect changes in risk factors, such as prenatal factors related to advanced maternal age, changing exposure to sex hormones, and environmental factors.[6,7] Furthermore, although the incidence of cancer has increased in all age groups, survival rates increased over the same time period in developed countries with access to cutting-edge resources.[5,6]

The ACCIS provides additional important demographic and epidemiologic data regarding cancer in children (aged birth-14 years) and adolescents (aged 15-19 years). Based on ACCIS data, the incidence of malignancies is higher in boys at all ages, with the exception of renal tumors, thyroid carcinomas, and melanomas, which are more common in girls.[5,6] Environmental factors (eg, exposure to radiation) and genetic factors (eg, familial syndromes) also play a role in determining the risk of cancer. The incidence and spectrum of specific pediatric malignancies also vary with small changes in age, and there are important differences in the patterns of malignancies observed at different ages (Table 2).[5] For example,

TABLE 2. Cancer Incidence by Age Group in Children Based on Data From the Automated Childhood Cancer Information System[5]

Age Group	Tumor Category
Infants (<1 y)	1) Sympathetic nervous system tumors
	2) Leukemias
	3) CNS tumors
	Others:
	Renal tumors
	Retinoblastomas
	Germ cell tumors
	Soft tissue sarcomas
	Lymphomas (rare)
	Bone tumors (rare)
Young children (1-4 y)	1) Leukemias
	2) CNS tumors
	3) Renal tumors
	Others:
	Sympathetic nervous system tumors
	Soft tissue sarcomas
	Lymphomas
	Hepatic tumors (rare)
	Bone tumors (rare)
School-age children (5-9 y)	1) CNS tumors
	2) Leukemias
	3) Lymphomas
	Others:
	Soft tissue sarcomas
	Bone tumors
	Hepatic tumors
Older school-age children or young adolescents (10-14 y)	1) Lymphomas
	2) Leukemias
	3) CNS tumors
	Others:
	Bone tumors
	Carcinomas
	Soft tissue sarcomas
	Germ cell tumors
	Retinoblastoma (rare)
	Renal tumors (rare)
	Liver tumors (rare)
Older adolescents (15-19 y)	1) Lymphomas
	2) Carcinomas
	3) Germ cell tumors
	Others:
	Leukemias
	CNS tumors
	Bone tumors
	Retinoblastoma (rare)
	Renal tumors (rare)
	Liver tumors (rare)

Abbreviation: CNS, central nervous system.

TABLE 3. Overall Frequency of Most Common Tumors in Children[5,7]

Tumor Category	Relative Frequency (% of All Childhood Malignancies)
Leukemias	31.3-44.8
CNS tumors	20.9-29.8
Lymphomas	10.1-15.5
Sympathetic nervous system tumors	8.0-10.0

Abbreviation: CNS, central nervous system.

lymphoid leukemias predominate between the ages of 2 and 3 years when they peak in occurrence, whereas the spectrum in adolescents more closely approximates that noted in adults, with a greater predominance of lymphomas and carcinomas. Lymphomas increase in incidence in the adolescent population due to the peak of Hodgkin lymphoma (HL) in this age group, whereas in adults non-Hodgkin lymphomas are more common. The carcinomas that predominate in children and adolescents include thyroid carcinomas, adrenocortical carcinomas, and nasopharyngeal carcinomas. It is interesting to note that approximately 40% of all malignancies in children and adolescents occur in children aged < 5 years, and as a consequence, these tumors are the most common overall in the pediatric population (Tables 2 and 3).[5,7]

Although to our knowledge few recent data regarding the incidence of specific subtypes of childhood cancer in developing countries exist, earlier GLOBOCAN data and data from smaller or disease-specific studies have suggested that geographic variability is considerable.[8] In contrast to developed countries, Burkitt lymphoma accounts for nearly one-half of all childhood cancers in equatorial Africa, whereas acute lymphoblastic leukemia is uncommon. Human immunodeficiency virus-related Kaposi sarcoma has increased in African children and in some countries, such as Uganda and Zimbabwe, is now more common than endemic Burkitt lymphoma. The incidence of hepatocellular carcinoma, which is associated with hepatitis B and C viral infections and is rare in developed countries, is higher in children in some Asian countries. Retinoblastoma is the most common solid tumor in some countries such as India, and there, as well as in countries such as Thailand, Uganda, Zimbabwe, and Costa Rica, exceeds neuroblastoma, which is more common than retinoblastoma in developed countries.

Based on ACCIS data, the overall 5-year survival rate for children with malignancies is approximately 65% to 75%, which represents a significant improvement over the 5-year survival rate of 50% reported during 1978 to 1982. Not surprisingly, the most frequent tumors (leukemias, central nervous system [CNS] tumors, sympathetic nervous system tumors, and soft tissue sarcomas) account for the majority of deaths.[5-7] Cancers with the greatest reduction in mortality include leukemias and lymphomas, in addition to retinoblastomas, hepatic tumors, and germ cell tumors, whereas CNS tumors and soft tissue sarcomas have demonstrated the lowest rate of change in mortality rates.[6] Based on data from the Surveillance, Epidemiology, and End Results program in the United States, advances in treatment have occurred at a faster pace for childhood malignancies than for those in adults and consequently have had a greater impact on prognosis in the pediatric age group.[7]

PEDIATRIC CYTOPATHOLOGY IN DIFFERENT PRACTICE SETTINGS

The types of malignancies that are represented in the cytology literature regarding pediatric tumors differ from the population-based statistics. The 2 most common childhood tumors are rarely represented in aspirates evaluated by cytopathologists. This includes leukemias that are typically evaluated by hematopathologists and CNS tumors that are rarely amenable to FNAB and moreover are usually examined by neuropathologists. Several other variables affect the types of pediatric tumors evaluated by FNAB. These include the practice setting (eg, academic vs community; resource-rich or resource-limited), type of hospital (eg, free-standing children's hospital or pediatric service within a university or community hospital), the types of clinicians that comprise the referral base (eg, pediatricians, oncologists, otolaryngologists, interventional radiologists), institutional access to cytologic evaluation of pediatric mass lesions (eg, involvement of cytopathologists, the presence of a pediatric FNAB service), and the overriding opinion of clinicians regarding diagnostic modality (eg, advocate of small biopsies or proponents of excisional biopsies). Thus, the percentage and types of malignancies evaluated by FNAB vary dramatically in different geographical regions and between institutions.

The majority of the world's pediatric population lives in resource-limited areas, in which the lack of access to major medical centers and the high incidence of

TABLE 4. Comparison of Pediatric Cytopathology in Resource-Limited Countries Versus the United States

	Resource-Limited Countries	United States
Percentage of worldwide pediatric population	Majority (>85%)	Minority
No. of pediatric FNABs	More	Fewer (variable)
Percentage of abdominal/pelvic FNABs	More abdominal/pelvic FNABs	Fewer abdominal/pelvic FNABs
Clinical acceptance	Greater	Less

Abbreviation: FNAB, fine-needle aspiration biopsy.

TABLE 5. Comparison of Pediatric Lesions Sampled in Head and Neck Versus Abdomen and Pelvis

	Head and Neck	Abdomen and Pelvis
Location	Superficial	Deep-seated
Type of FNAB	Palpation-guided FNAB or image-guided FNAB	Image-guided FNAB
Types of tissue specimens	Mostly lymph nodes	Mostly solid organ masses
Benign vs malignant	Benign >> malignant	Malignant>>benign

Abbreviation: FNAB, fine-needle aspiration biopsy.

infectious diseases that can be quickly diagnosed by cytology to expedite treatment promote the use of FNAB. In these regions, acceptance of FNAB as a primary diagnostic modality and willingness to treat based on the cytologic diagnosis are more common than in resource-rich countries. This stands in stark contrast to the United States, in which there is underuse of FNAB in pediatric populations and trepidation with regard to treatment based on a cytologic diagnosis, which are compounded and perpetuated by Children's Oncology Group (COG) protocols that emphasize the need for histological sampling. Differences in the approach to the diagnosis of Wilms tumor illustrate this dichotomy.[9] The International Society of Paediatric Oncology protocols for the treatment of Wilms tumor use preoperative chemotherapy as the initial therapy and thus staging is performed on the posttreatment resection specimen. In this setting, FNAB has proven to be accurate for the diagnosis of renal tumors and as a basis for directing treatment.[10] In contrast, the COG protocols base the staging of Wilms tumor on the primary prechemotherapy resection specimen and emphasize the need for histological evaluation before treatment.[9]

In the limited publications on cytologic diagnosis of pediatric tumors available in the United States, the majority of FNABs are from lesions of the head and neck, lymph nodes, and soft tissue.[11-15] Benign diagnoses outnumber malignant diagnoses, with only 10% to 40% of cases being malignant. The majority of malignancies reported include lymphomas, sarcomas, thyroid carcinomas, and small round cell tumors. Abdominal malignancies represent a small minority of the tumors in these series. In contrast, in a study that included 290 FNABs from resource-poor areas, 77% of the diagnoses were malignant.[4] In this study, the most common malignancies were Wilms tumor, lymphomas, rhabdomyosarcomas, and neuroblastoma. FNAB resulted in the precise subtyping of the tumor that allowed for the initiation of treatment in 76% of cases, including neoadjuvant treatment before surgery.[4] Furthermore, this and another large study reported that the sensitivity and specificity of FNA were 97% to 98% and 93% to 97%, respectively.[3,4] A study from Argentina that examined 899 FNA specimens from patients aged < 20 years and a smaller study from Spain demonstrated that > 50% of the cases were malignant.[3,16] The majority of these malignancies were from abdominal masses. Differences between aspirates from the United States and those from resource-limited settings are summarized in Tables 4 and 5.

In the United States, the National Cancer Institute supports the COG, a cooperative group that conducts clinical trials, as well as research on the biology, risk factors, and outcomes of childhood cancers. Given that 90% to 95% of children aged < 15 years with a newly diagnosed malignancy are seen at COG institutions, pathologists and cytopathologists at these institutions typically have more experience in diagnosing these tumors and greater resources with which to perform the necessary ancillary studies compared with institutions that are not affiliated with COG.[17] However, COG protocols are based on histologic diagnosis and associated biologic

studies require frozen or formalin-fixed tissue from the primary tumor and, when available, metastatic and recurrent tumors. These requirements serve as powerful deterrents to the use of FNAB as a primary diagnostic modality. However, as the use of small biopsies increases and rising numbers of clinically validated molecular tests shift the focus from the precise classification of disease to the identification of biomarkers for targeted therapies, the use of FNAB for the diagnosis of suspected pediatric malignancies may increase.[18]

DIFFERENTIAL DIAGNOSES

Childhood tumors are frequently classified based on morphology, rather than site of origin, as is typically used for adult tumors.[1,15,19] This reflects the differences in the types of tumors noted in these populations and, from a practical perspective, provides a useful framework for the pathologic evaluation of mass lesions in children and adolescents. Common morphologic patterns include inflammatory, epithelial/epithelioid, cystic, spindle cell, clear cell, small round cell, and large cell/pleomorphic. Differential diagnostic considerations using this pattern-based approach are summarized in Table 6 and the cytologic features of the more commonly encountered entities in each category are discussed (Fig. 1)

Inflammatory Pattern

An inflammatory pattern most frequently represents a benign process, such as reactive lymphoid hyperplasia, infection, or inflammation involving the aspirated tissue or organ, but is also characteristic of Langerhans cell histiocytosis (LCH) and HL, and may rarely mask other malignancies (Figs. 1A-1D).[15,19] Aspirates from reactive lymphoid hyperplasia are highly cellular and comprised of a polymorphous population of lymphocytes, dominated by small mature lymphocytes but spanning a spectrum from small lymphocytes to immunoblasts, with varying numbers of interspersed plasma cells and tingible body macrophages (Fig. 1A). A high-grade lymphoma should be excluded, particularly in the setting of numerous tingible body macrophages or a monomorphic intermediate-to-large cell population. Depending on the inciting stimulus, eosinophils may also be present. These features are also characteristic of HL, in which the malignant cells occur in a heterogeneous background with varying numbers of interspersed histiocytes and eosinophils. Classic and/or variant Reed-Sternberg cells in the appropriate background are diagnostic of HL, but may comprise a minority of cells and be overlooked or underrepresented on cell blocks (Fig. 1D). Fragments of metachromatic stroma are characteristic of the nodular sclerosis subtype of classic HL, which is the most common variant in the pediatric population, and sarcoid-like granulomas and/or necrosis may also be present. Although these features provide clues to the diagnosis, they are nonspecific. Granulomatous lymphadenitis is characteristic of infections due to atypical mycobacteria, *Mycobacterium tuberculosis*, Bartonella, and fungal and other organisms, as well as other processes encountered in the pediatric population, such as chronic granulomatous disease, sarcoidosis, and rheumatoid arthritis. Similarly, necrosis is encountered in aspirates from abscesses or acute suppurative infectious processes, as well as other tumors. Immunohistochemical (IHC) stains are useful for confirming the diagnosis of HL. Classic and variant Reed-Sternberg cells are positive for CD30, CD15, MUM-1, and fascin, and are negative for CD45 and ALK-1. L and H (so-called popcorn) cells, which are characteristic of nodular lymphocyte-predominant HL, a rare variant that is infrequently encountered in the pediatric population, are positive for CD45, CD20, and CD79a and negative for CD30 and CD15. In specimens of HL, flow cytometry reveals a reactive pattern, and thus does not contribute to the diagnosis, aside from excluding the possibility of a non-Hodgkin lymphoma in difficult cases. It is interesting to note that inflammatory cells can also be present in other malignancies, such as a lymphohistiocytic or granulomatous infiltrate in seminoma, and thus the presence of inflammation alone does not exclude the need to thoroughly search for neoplastic cells. Furthermore, certain leukemias, such as myeloid leukemias and myeloid sarcomas, can mimic an acute inflammatory process and should be considered, particularly in the appropriate clinical scenario.

Aspirates from LCH, a monoclonal neoplastic proliferation of Langerhans cells that is classified among the histiocytic neoplasms in the 2008 World Health Organization classification,[20] also have an inflammatory pattern. The Langerhans cells are present in a background of mixed inflammation composed of variable numbers of lymphocytes, eosinophils, neutrophils, and mononuclear and multinucleated non-Langerhans histiocytes. Langerhans cells are characterized by pale moderately abundant cytoplasm and folded ovoid nuclei with inconspicuous

TABLE 6. Pattern-Based Approach to the Differential Diagnosis of Tumors in Pediatric Cytopathology

Cytological Pattern	Differential Diagnosis
Inflammatory	Acute suppurative lymphadenitis
	Abscess
	Granulomatous inflammation or lymphadenitis
	Infection
	Langerhans cell histiocytosis
	Hodgkin and non-Hodgkin lymphoma
Epithelial/epithelioid	Thyroid nodules (hyperplastic, neoplastic)
	Benign and malignant salivary neoplasms
	Nasopharyngeal carcinoma
	Fibroadenoma
	Pilomatricoma
	Benign and malignant hepatic neoplasms
	Adult-type carcinomas
	Epithelioid sarcoma
Cystic	Developmental cysts
	Parathyroid cyst
	Benign thyroid cyst/colloid nodule
	Papillary thyroid carcinoma with cystic change
	Lymphangioma
	Benign bone cyst
	Ganglion or synovial cyst
	Renal neoplasms with cystic change
Clear cell	Perivascular epithelioid cell tumor (PEComa)
	Renal cell carcinoma, including those associated with Xp11.2 (*TFE*) translocations
	Germ cell tumors (yolk sac tumor, seminoma)
	Hemangioblastoma
	Xanthogranulomatous pyelonephritis
	Lipomatous lesions (fat necrosis, neoplasms)
	Clear cell sarcoma of soft tissue or kidney
	Clear cell change in a variety of tumors
Spindle cell	Peripheral nerve tumors
	Spindle cell sarcomas (synovial sarcoma, spindle cell rhabdomyosarcoma, infantile fibrosarcoma)
	Nodular fasciitis
	Fibromatosis
	Fibrous hamartoma of infancy
Small round cell	Hematolymphoid proliferations (reactive, neoplastic)
	Ewing sarcoma/PNET
	Rhabdomyosarcoma
	Small cell osteosarcoma
	Desmoplastic small round cell tumor
	Neuroblastoma
	Other blastomas: medulloblastoma, retinoblastoma, nephroblastoma, hepatoblastoma, pancreatoblastoma
	Pilomatricoma
Large cell	Hematolymphoid proliferations (Hodgkin, anaplastic, and diffuse large B-cell lymphomas)
	Osteosarcoma (conventional)
	Rhabdomyosarcoma and other rhabdoid tumors
	Alveolar soft part sarcoma
	Germ cell tumors
	Malignant melanoma
	Hepatocellular carcinoma
	Ganglioneuromas/ganglioneuroblastomas
	Granular cell tumor
	Granulomatous inflammation
	Langerhans cell histiocytosis

Abbreviation: PNET, primitive neuroectodermal tumor.

nucleoli (Fig. 1C). The presence of eosinophils is a useful clue to the diagnosis, particularly in aspirates with few Langerhans cells. Ultrastructurally, Langerhans cells have Birbeck granules; however, IHC stains are more often used to confirm the diagnosis. Cells are positive for CD1a, S-100, and langerin (CD207), and may aberrantly express CD68. Although activated macrophages may express S-100, in contrast to Langerhans cells they are positive for CD68 and CD163, and negative for CD1a and langerin.

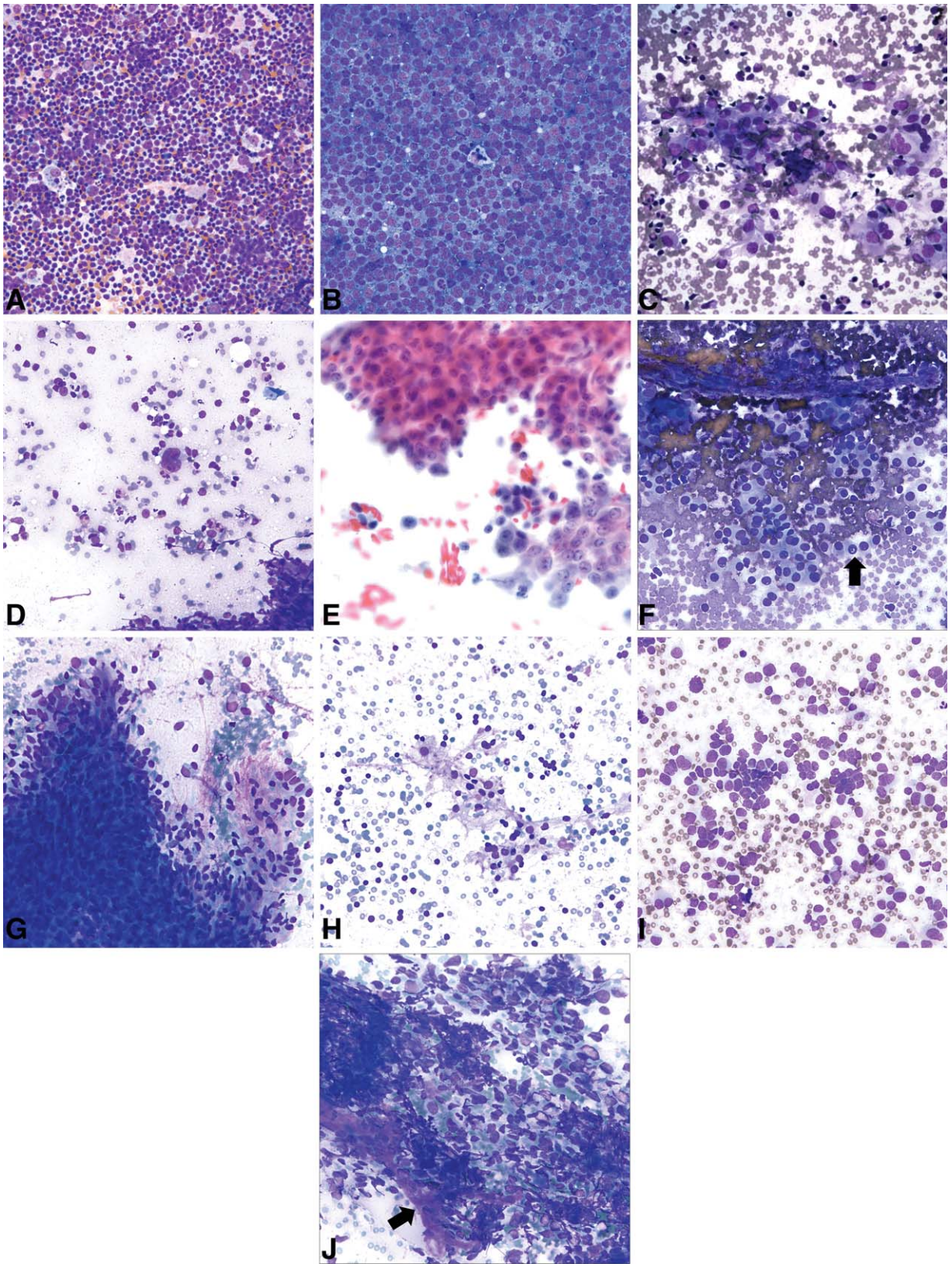

Figure 1.

Epithelial/Epithelioid Pattern

A minority of pediatric malignancies present with an epithelial/epithelioid pattern, reflecting the relative rarity of carcinomas in this population compared with adults.[3,4,15,19] An epithelial pattern is observed in thyroid, salivary, and hepatic neoplasms; nasopharyngeal carcinomas; and the rare, adult-type carcinomas arising in children and adolescents. Some of these tumors, such as thyroid carcinomas, may be associated with a particular genetic association or familial syndrome and therefore be more likely to be diagnosed in young patients. This includes the occurrence of the cribriform morular variant of papillary thyroid carcinoma in the setting of familial adenomatous polyposis syndrome, and medullary thyroid carcinoma in the setting of multiple endocrine neoplasia syndromes.[21,22] However, in general, the cytologic features of these epithelial malignancies are identical irrespective of age and are well characterized in the standard textbooks on cytopathology. Therefore, with the exception of hepatoblastoma, a rare tumor predominantly of young children, these entities will not be discussed further.

Hepatoblastoma is subdivided into epithelial and mixed epithelial-mesenchymal subtypes, and aspirates tend to be cellular. Fetal and/or embryonal cells comprise the predominant epithelial elements, whereas a minority have small cell or macrotrabecular patterns. Fetal cells are smaller than hepatocytes, and have moderate amounts of granular or clear cytoplasm and round central nuclei (Fig. 1E). Embryonal cells are smaller and more primitive with scant amphophilic cytoplasm, and irregular nuclei with coarse chromatin and prominent nucleoli. Mesenchymal elements are present in nearly one-half of the tumors, and in some of these, teratoid elements are also observed.[23-26] Extramedullary hematopoiesis is common. Using IHC, fetal and embryonal epithelial cells are positive for α-fetoprotein, glypican-3, glutamine synthetase, and nuclear β-catenin. Fetal and, to a lesser extent, embryonal cells are positive for hepatocyte paraffin 1, cytokeratins 8 and 18 (Cam 5.2), and carcinoembryonic antigen. Distinction between the fetal pattern of hepatoblastoma and well-differentiated hepatocellular carcinoma may be difficult in cytologic preparations. The cells of fetal hepatoblastoma are typically smaller and more uniform than those of hepatocellular carcinoma and are more likely to demonstrate nuclear staining for β-catenin. If present, embryonal or mesenchymal elements also provide a clue to the correct diagnosis. However, in young children, clinical correlation is often the most useful tool for distinguishing these entities, given the extreme rarity of hepatocellular carcinoma in this age group in the absence of a predisposing metabolic disorder.

Cystic Pattern

The vast majority of pediatric masses resulting in a cystic pattern on FNAB are benign processes, such as developmental and other cysts, colloid nodules, and lymphatic malformations. However, cystic changes may occur in

Figure 1. Representative examples in the pattern-based approach to pediatric cytopathology are shown, specifically (A-C) inflammatory, (D) large cell, (E) epithelial, (F) cystic, (G) spindle cell, (H) clear cell, and (I-J) small round cell patterns. (A) Reactive lymphoid hyperplasia is shown. Aspirates demonstrate a heterogeneous lymphoid population with scattered tingible body macrophages and an absence of eosinophilia or Reed-Sternberg cells (Diff-Quik, × 400). (B) Burkitt lymphoma is shown. These aspirates reveal a monomorphic population of intermediate-sized lymphocytes with mitoses and tangible body macrophages. There is a lack of heterogeneity and a shift toward more intermediate-sized cells, in comparison with panel A (Diff-Quik, × 400). (C) Langerhans cell histiocytosis is shown. Aspirates reveal an inflammatory background with increased eosinophils, in addition to intermediate-sized histiocytic cells with abundant cytoplasm and cleaved nuclei (Diff-Quik, × 400). (D) Classic Hodgkin lymphoma is shown. Classic Hodgkin lymphomas have a heterogeneous lymphoid population and an increase in eosinophils, with Reed-Sternberg cells that are usually binucleated with prominent nucleoli (Diff-Quik, × 400). (E) Hepatoblastoma is shown. Aspirates show fetal cells, which tend to be epithelioid cells that are smaller and more uniform than hepatocytes, in addition to embryonal cells (H & E, × 400). (F) Papillary thyroid carcinoma is shown. Aspirates from primary or metastatic papillary thyroid carcinomas can demonstrate cystic changes with histiocytes and/or colloid. This example shows follicular epithelial cells with intranuclear cytoplasmic inclusions (arrow) and grooves in a background of watery colloid (Diff-Quik, × 400). (G) Synovial sarcoma is shown. This spindle cell tumor demonstrates cellular aspirates with monomorphic-appearing spindle cells and interlacing metachromatic material (Diff-Quik, × 400). (H) Renal cell carcinoma associated with the transcription factor E3 (*TFE3*) gene translocation is shown. This clear cell tumor shows abundant pale cytoplasm and round nuclei, in addition to stripped nuclei (Diff-Quik, × 400). (I) Neuroblastoma is shown. Neuroblastoma typically yields cellular aspirates with cells that have dark round nuclei, scant cytoplasm, nuclear molding, and clustering with occasional rosette formation. No lymphoglandular bodies are noted (Diff-Quik, × 400). (J) Small cell osteosarcoma is shown. Aspirates from this bone lesion show a small round cell tumor with occasional metachromatic osteoid material (arrow) (Diff-Quik, ;× 400).

TABLE 7. Distinguishing Features of Selected Pediatric Small Blue Cell Tumors

Tumor	Cytomorphology	Immunophenotype	Genetics
Wilms tumor	Blastema +/-epithelial component +/- stroma, rarely, anaplasia	+ WT1; + EMA, cytokeratin (epithelial component); - Synaptophysin, chromogranin	Mutations of *WT1, WT2*
Neuroblastoma	Neuropil, rosettes, +/- ganglion cells, +/- schwannian stroma, +/- calcification	+ Synaptophysin, chromogranin, CD56, PgP9.5; - S-100, CD99, desmin, myogenin, lymphoid markers	+/- *N-MYC* amplification
Rhabdomyosarcoma	Rhabdomyoblastic differentiation subtle to obvious+/- floret cells, +/- strap cells	+ Myogenin, myoD1, desmin; - TLE1; +/− aberrant CD99, cytokeratin, EMA, neural markers	Alveolar subtype: t(2;13)(q35;q14), t(1;13)(p36;q14)
Ewing sarcoma/PNET	+/- Rosettes, +/-neuropil,+/- Tigroid background	+CD99, FLI-1;+/- synaptophysin, PgP9.5, CD56;- Desmin, myogenin, CD45, TLE-1, EMA, cytokeratins	t(11;22)(q24;q12) in >90%; t(21;22)(q12;q12), t(2;22)(q33;q12), others rare
Synovial sarcoma (round cell)	+/- Metachromatic stroma, +/- calcifications	+ TLE-1, EMA; +/- cytokeratin, CD99; - myogenin, myoD1, desmin	t(X;18)
Small cell osteosarcoma	Osteoid (often scant or absent)	+/- S-100, osteonectin, osteocalcin, CD99;- FLI-1, myogenin, neural markers	*EWS* rearrangements absent
Lymphoid malignancies	Morphology varies with type; lymphoglandular bodies	Varies with lineage and type (B-cell, T-cell);+TdT (lymphoblastic lymphoma)	Burkitt: *MYC* translocations, t(8;14)(q24;q32) and less commonly, t(2;8)(p12;q24), t(8;22)(q24;q11)

Abbreviations: +, positive; -, negative; EMA, epithelial membrane antigen; *EWS*, Ewing sarcoma gene; FLI-1, friend leukemia integration 1 transcription factor; PNET, primitive neuroectodermal tumor; TdT, terminal deoxynucleotidyl transferase; TLE-1, transducin-like enhancer of split 1; *WT1*, Wilms tumor 1; *WT2*, Wilms tumor 2.

papillary thyroid carcinoma and, less frequently, other pediatric malignancies, and may result in a false-negative diagnosis unless these entities are considered (Fig. 1F). Aspirates comprised of cyst contents or fluid may have obscuring inflammatory elements or harbor few or no malignant cells, thereby making an accurate diagnosis difficult or impossible. When the clinical presentation and/or imaging studies suggest a malignant neoplasm, the processing of any residual cyst fluid is essential before rendering a cytologic diagnosis. Failure to sample lesional tissue surrounding a cyst cavity is a well-recognized source of false-negative diagnoses and thus correlation with the radiographic findings is essential. In addition, cystic lesions with a predominance of squamous cells should be approached differently in children compared with adults. In children, these findings likely represent developmental cysts with squamous lining or squamous metaplasia and carcinoma is highly unlikely, whereas in adults the possibility of metastatic squamous cell carcinoma with cystic changes must be excluded. In addition, germ cell tumors with a teratomatous component containing squamous epithelium may also give rise to a cystic neck mass in adolescents, and should be considered in the appropriate clinical setting.

Spindle Cell Pattern

A spindle cell pattern is characteristic of FNAB specimens from a variety of common and uncommon pediatric mesenchymal proliferations. These include nonneoplastic tumefactions, such as nodular fasciitis and traumatic neuroma; benign neoplasms, such as fibroma and schwannoma; and malignancies, such as synovial sarcoma, malignant peripheral nerve sheath tumor (MPNST), and spindle cell rhabdomyosarcoma. Furthermore, some neoplasms, such as hepatoblastomas and Wilms tumors, may be morphologically heterogeneous and therefore a spindle cell pattern may predominate in tumors that are not typically associated with a pure spindle cell pattern. Overall, reactive and benign neoplastic proliferations comprise the majority of pediatric spindle cell lesions; however, this discussion will focus on the cytologic features and differential diagnosis of selected malignant tumors.

Considerable overlap exists in the cytomorphologic features of pediatric spindle cell sarcomas. Aspirates vary in cellularity, often reflecting the grade of the tumor and the presence or absence of collagenized stroma. Thus, FNAB specimens from high-grade tumors without fibrotic stroma are typically highly cellular, whereas those

from some low-grade tumors with dense collagenized stroma may be less cellular. Low-grade tumors are comprised of relatively bland cells and may be difficult to distinguish from their benign counterparts (eg, low-grade MPNST vs schwannoma). In contrast, high-grade tumors have poorly differentiated malignant cells, necrosis, and/or mitoses. Despite the overlap between spindle cell tumors, a specific cytologic diagnosis is often possible based on characteristic morphologic features and ancillary studies, including IHC, fluorescence in situ hybridization (FISH), and cytogenetics.

Monophasic spindle synovial sarcoma is a high-grade tumor characterized by a relatively monomorphous population of short spindle cells with scant cytoplasm and elongated nuclei with coarse hyperchromatic nuclei and ≥ 1 nucleoli. Dense metachromatic stroma is often present but may be scant and subtle (Fig. 1G). If present, epithelial cells in clusters or glandular arrays strongly suggest a diagnosis of biphasic synovial sarcoma, with the caveat that spindle and epithelioid elements may be also present in gastrointestinal stromal tumor (GIST) or, rarely, MPNST. The spindle cells of synovial sarcoma are positive for vimentin, transducing-like enhancer of split 1 (TLE-1), and at least focally for epithelial membrane antigen (EMA); variably positive for CD99 (membrane and/or cytoplasmic) and S-100; and negative for CD34 and desmin. Focal positivity for cytokeratins (CK) 7 and 19 may also be present, but is less frequent than staining for EMA. Approximately 85% of synovial sarcomas have a specific translocation, t(X;18), resulting in an *SYT-SSX* fusion, detectable by FISH, reverse transcriptase-polymerase chain reaction (RT-PCR), or cytogenetics.

In contrast to synovial sarcoma, high-grade MPNST is characterized by pleomorphic spindle cells with hyperchromatic, elongate, wavy, or curved nuclei with variably tapered ends and irregular nuclear membranes. Varying numbers of bizarre tumor giant cells may also be observed. When present, delicate, fibrillary, metachromatic stroma and nuclear palisading provide important clues to the diagnosis. In contrast to the strong diffuse staining observed in benign schwannoma, S-100 is only focally positive in MPNST and is negative is up to 50% of tumors. EMA, cytokeratin, CD99, desmin, and actins are usually negative. Characteristic cytogenetic abnormalities are absent. In the pediatric population, the vast majority of MPNSTs arise in the setting of neurofibromatosis type 1 and thus the clinical history provides an important clue to the correct diagnosis.

Spindle cell rhabdomyosarcoma is characterized by spindle cells resembling those of adult-type fibrosarcoma, leiomyosarcoma, or MPNST arrayed singly and in irregular aggregates or sheets, often with a fascicular pattern. The cytomorphology varies between and sometimes within tumors, and ranges from relatively bland to highly pleomorphic. When present, cells with abundant eosinophilic cytoplasm with cross-striations are an important clue to the diagnosis. Nuclear positivity for myogenin, which is typically focal, distinguishes spindle cell rhabdomyosarcoma from other entities in the differential diagnosis. In addition, the cells are positive for desmin and muscle-specific actin and negative for S-100, CD34, EMA, cytokeratin, and TLE-1. Characteristic cytogenetic abnormalities are absent.

GIST is characterized by highly cellular aspirates composed of spindle cells or less often epithelioid cells or a combination of the two. It is interesting to note that epithelioid features are more common in GIST occurring in the pediatric population than in adults, and their presence provides a helpful clue to the correct diagnosis. The spindle cells are relatively uniform with delicate cytoplasm and elongate, blunt-ended, hyperchromatic nuclei. Paranuclear cytoplasmic vacuoles may be present. Dense hyaline or myxoid stroma may be evident in some cellular aggregates or in the background. When present, epithelioid cells appear as large polygonal cells with abundant eosinophilic to clear cytoplasm and central round or oval nuclei. The tumor cells are usually positive for CD117 (c-kit), DOG-1, and CD34; variably positive for smooth muscle actin; and negative for S-100 and desmin. GISTs are characterized by mutations in *KIT*, or less frequently platelet-derived growth factor receptor, α polypeptide (*PDGFR-A*) or *BRAF*.

A variety of low-grade spindle cell malignancies occur in the pediatric population, and although a specific diagnosis may be possible in some cases, distinction between these tumors and benign neoplastic or reactive processes may be difficult or impossible in cytologic preparations. In practice, this distinction may not be critical, nor is a specific diagnosis usually essential. However, it is important to recognize features such as increased cellularity, mild cytologic atypia, and increased mitotic activity, which may be suggestive of a low-grade spindle cell malignancy, and clearly communicate these findings to the

clinician. When a definitive cytologic diagnosis is not possible and the differential diagnosis includes low-grade malignancies and benign processes, a biopsy may help to clarify the diagnosis and thereby ensure adequate resection of a malignant tumor, while avoiding overly aggressive treatment of a benign lesion.

Clear Cell Pattern

Pediatric tumors with a predominance of clear cells may be diagnostically challenging due to the morphologic overlap with benign histiocytic lesions, and the low nuclear-to-cytoplasmic ratio in the tumor cells. This group includes perivascular epithelial tumors (PEComas), germ cell tumors (yolk sac tumors, seminomas), clear cell sarcomas, and carcinomas with clear cell change (eg, renal cell carcinoma [RCC]). Benign entities in the differential diagnosis include xanthogranulomatous proliferations and adipocytic lesions.

The PEComa family of neoplasms, characterized by cells with myomelanocytic differentiation, includes angiomyolipomas, lymphangiomyomatosis, and clear cell "sugar" tumor of the lung. Many of these lesions are initially misdiagnosed, and have been reported in a wide variety of locations. In children, these may occur sporadically or in association with tuberous sclerosis. Cytological features include epithelioid cells with clear cytoplasm, usually near vessels, with more discohesive spindle cells and multinucleated giant cells. The cells are typically positive for melanocytic markers (HMB-45, MelanA) and muscle markers (smooth muscle actin), but are negative for cytokeratin, CD117/C-kit, and CD34. PEComa must be distinguished from clear cell sarcoma of soft tissue, which is also composed of epithelioid and spindle cells with clear or granular cytoplasm that is positive for HMB-45 and MelanA but negative for muscle markers.

Germ cell tumors, particularly yolk sac tumors and seminomas, may also have abundant clear cytoplasm. Yolk sac tumors have a variety of histological patterns, and therefore may vary from cohesive to discohesive on FNAB, but generally have moderately abundant clear cytoplasm. Cytoplasmic and/or extracellular hyaline globules are an important clue to the diagnosis. In seminoma, the tumor cells appear discohesive with round central nuclei, prominent nucleoli, and pale cytoplasm. Due to the presence of glycogen, the disrupted cytoplasm imparts a "tigroid" appearance to the background on air-dried slides stained with modified Romanowsky stain. Lymphocytes and granulomas are often present. Yolk sac tumors typically are positive for α-fetoprotein, cytokeratin, and glypican-3, and negative for CD117/C-kit and Oct3/4, whereas the opposite pattern is seen in seminomas.

Clear cell sarcoma of the kidney (CCSK) is a rare, deceptively bland tumor that typically occurs in children aged < 5 years. In aspirates, CCSK appears as small spindle or epithelioid cells arrayed singly or in aggregates with transgressing blood vessels. The cells have clear cytoplasm and nuclei with distinct grooves and fine chromatin. CCSKs are positive for vimentin, but typically negative for nearly all other stains, including cytokeratin, S-100, CD99, desmin, WT-1, and synaptophysin.

A clear cell phenotype is often observed in both conventional and Xp11.2 (transcription factor E3 [*TFE*] gene) translocation-associated RCC. The latter variant is characterized by translocations of *TFE3* on chromosome Xp11.2, resulting in overexpression of TFE3, which can be detected by IHC.[27] These tumors are typically observed in children and young adults, and although only approximately 5% of pediatric renal tumors are RCC, greater than one-third of these are Xp11.2 translocation RCC.[28] The key cytological features of these tumors include clear cells with loose cohesion or in papillary fragments and numerous stripped nuclei (Fig. 1H). Cells have voluminous cytoplasm with punched-out, discrete cytoplasmic vacuoles and central nuclei. Hyalinized metachromatic globules, psammoma bodies, and thin transgressing vessels may also be noted. RCCs associated with *TFE3* translocations tend to be negative or only focally positive for epithelial markers, such as cytokeratins and EMA, and are usually negative for vimentin, in contrast to conventional RCC. Nuclear staining for TFE3 by IHC is a helpful marker for RCC associated with *TFE3* translocations, and a FISH break-apart probe for the *TFE3* gene can also confirm the presence of a translocation in these tumors.[27,28]

Small Round Cell Tumors

Small round cell tumors (SRCTs) are the most common group of pediatric malignancies and encompass a broad spectrum of tumors, arising from hematolymphoid, mesenchymal, neuroepithelial, neural crest, epithelial, and primitive blastemal cells. As with spindle cell tumors, considerable overlap exists in the cytomorphologic features of SRCTs. FNAB specimens are typically highly cellular and are composed either of predominantly single cells (eg,

lymphomas, Ewing sarcoma) or discohesive cellular aggregates with single cells in the background (eg, neuroblastoma, Wilms tumor). Necrosis and mitotic figures, including abnormal forms, are often present. Despite the overlap between SRCTs, a specific cytologic diagnosis is often possible based on characteristic morphologic features and ancillary studies, including IHC, FISH, RT-PCR, and cytogenetics.

Wilms tumors (nephroblastomas) typically have a blastemal component (nearly 100% of cases) and an epithelial component (approximately 70% of cases), whereas a mesenchymal component is less frequent (approximately 20% of cases).[25] In FNABs, the blastemal component is characterized by small cells with scant cytoplasm and round or irregular, often molded nuclei with coarse chromatin, which are arrayed as discohesive aggregates and single cells. In contrast, the epithelial elements appear as clusters or tubules composed of cells with moderate to abundant cytoplasm. When present, the mesenchymal component is composed of bland spindle cells in a background of metachromatic collagenous or myxoid matrix. Anaplasia, defined by the triad of nuclei at least 3 times the size of adjacent tumor nuclei, hyperchromasia, and multipolar mitotic figures, is observed in $< 5\%$ of Wilms tumors. Anaplasia may be focal and, as a consequence, not sampled in FNAB. However, its presence correlates with poor prognosis and therefore, it is important to recognize, particularly in the setting of preoperative chemotherapy. Wilms tumors are usually positive for WT1 and negative for chromogranin and synaptophysin, and retain INI-1. The blastemal component is variably positive for cytokeratin and desmin, and the epithelial component is positive for EMA and cytokeratin. When rhabdomyoblastic differentiation is present, the mesenchymal component is positive for desmin and myogenin. It is important to note that nephrogenic rests and nephroblastomatosis are indistinguishable from Wilms tumor in cytologic preparations, and therefore correlation with imaging studies is important to confirm the diagnosis of Wilms tumor.

In contrast to Wilms tumors, neuroblastomas appear more monomorphic and are composed of cells with round nuclei with fine stippled chromatin and small nucleoli (Fig. 1I). Neuropil, which appears as metachromatic fibrillary matrix; rosettes; and immature to mature ganglion cells are variably present, depending on the degree of differentiation, and are diagnostic of neuroblastoma.[25] Ancillary studies are essential for the diagnosis of undifferentiated neuroblastoma and help to confirm the diagnosis when neuropil and/or ganglionic differentiation are present. The tumor cells are usually positive for synaptophysin, chromogranin, and CD56, and negative for S-100, CD99, desmin, myogenin, and lymphoid markers. FISH is useful for the assessment of *N-MYC* amplification, which is used for risk stratification and treatment. The mitotic-karyorrhectic index is important for determining favorable or unfavorable histology, another feature used in risk stratification, but cannot be assessed in cytologic preparations.

Aspirates from rhabdomyosarcoma are characterized by relatively monotonous round cells. In alveolar rhabdomyosarcoma (ARMS), the cells are often larger than those of embryonal rhabdomyosarcoma (ERMS), Ewing sarcoma/primitive neuroectodermal tumor (PNET), or synovial sarcoma, and have round nuclei with coarse chromatin and ≥ 1 conspicuous nucleoli. Small cells with eccentric eosinophilic cytoplasm and occasional large multinucleated cells with eosinophilic cytoplasm and peripherally placed nuclei (floret or wreath cells) may be identified in ARMS, but cells with cross-striations are uncommon. Fragments of fibrous stroma may be present. In contrast, ERMS is composed of smaller round cells with round or oval hyperchromatic nuclei with fine chromatin and inconspicuous nucleoli. Elongate cells with cross-striations may be present, and in some cases are numerous, whereas floret cells are usually rare. Fragments of myxoid stroma may be present. Nuclear positivity for myogenin, which is typically strong and diffuse in ARMS and focal in ERMS, distinguishes rhabdomyosarcomas from other entities in the differential diagnosis. In addition, the cells are positive for desmin and muscle-specific actin, and usually negative for chromogranin, synaptophysin, CD99, EMA, cytokeratin, and TLE-1. It is interesting to note that aberrant staining for cytokeratins and neural markers is observed in a significant minority of ARMS cases. Translocations involving *FOXO1* (forkhead box protein O1) on chromosome 13 and either *PAX3* (paired box 3) or *PAX7* on chromosomes 2 and 1, respectively, are present in approximately 80% of ARMS but not ERMS, and can be detected by FISH, RT-PCR or cytogenetics. In the absence of a translocation, FNAB does not reliably distinguish between ARMS and ERMS.

Ewing sarcoma/PNET is composed of cells with scant pale cytoplasm and round to oval nuclei with fine pale chromatin and inconspicuous nucleoli. Homer-

Wright rosettes may be present, depending on the degree of differentiation of the tumor. Crush artifact is often present. A tigroid background may be evident in air-dried smears stained with modified Romanowsky stains, but matrix is absent. Ewing sarcoma/PNET is usually positive for CD99 and FLI-1; variably positive for PgP9.5, synaptophysin, and CD56; and negative for desmin, myogenin, CD45, TLE-1, EMA, and cytokeratins. Greater than 90% of Ewing sarcomas/PNETs have a characteristic translocation, t(11;22)(q24;q12), which results in fusion of *FLI-1* and *EWSR1*. Other translocations involving *EWSR1* or *ERG* occur in another 5% of cases.

Round cell, poorly differentiated synovial sarcoma is composed of cells with round to ovoid hyperchromatic nuclei with irregular membranes, coarse chromatin, and prominent nucleoli. As with other synovial sarcomas, metachromatic fibrous stroma and calcifications may be present. Round cell, poorly differentiated synovial sarcoma has the same immunophenotype and specific translocation, t(X;18), as other variants.

Small cell osteosarcoma is an extremely rare variant of osteosarcoma, which may be confused with Ewing sarcoma/PNET. The cells are the same size as or somewhat larger than those of Ewing sarcoma/PNET and have scant cytoplasm and round or oval nuclei with granular chromatin and small nucleoli. Short spindle cells may also be present. The presence of osteoid is diagnostic, but may be scant or absent in cytologic specimens (Fig. 1J). Small cell osteosarcomas are often positive for CD99, but have no characteristic cytogenetic abnormalities.

Desmoplastic small round cell tumor is another round cell tumor affecting young patients, and typically presents with abdominal pain and distention in adolescent and young men. These are typically aggressive mesenteric or pelvic masses that may undergo image-guided FNA or core needle biopsy, and demonstrate uniform oval-to-round nuclei without conspicuous nucleoli and scant cytoplasm. Mitoses and spindled or rhabdoid-like cells are frequently noted. Necrosis and fragments of metachromatic desmoplastic stromal material can also be observed. These tumors are unique in that they are positive for epithelial (eg, cytokeratin and EMA), neural (eg, neuron-specific enolase), and muscle (eg, desmin) markers. The desmin staining is also typically perinuclear and dot-like. FISH studies have shown a t(11;22)(p13;q12) translocation between *EWS* and *WT1*, which is similar but not identical to that noted in Ewing sarcoma/PNET.

Lymphoblastic and Burkitt lymphomas comprise the vast majority of pediatric lymphomas in the differential diagnosis of SRCTs. Lymphoblastic lymphoma is comprised of small to medium-sized cells with scant cytoplasm and round or convoluted nuclei with immature fine chromatin and nucleoli that vary from inconspicuous to multiple. Both T- and B-lymphoblastic lymphomas are typically positive for terminal deoxynucleotidyl transferase (TdT), which helps to distinguish them from other lymphomas. Flow cytometry and/or immunocytochemistry are used to further classify the cells as T cell or B cell in origin. Burkitt lymphoma is composed of medium-sized cells with scant cytoplasm with multiple small vacuoles and round nuclei with clumped chromatin and multiple small nucleoli. Mitoses and apoptoses are usually readily apparent, and numerous tingible body macrophages are present in the background (Fig. 1B). Nuclear positivity for Ki-67 is observed in nearly 100% of cells. Burkitt lymphoma has a mature B-cell phenotype by flow cytometry and immunocytochemistry and, in contrast to lymphoblastic lymphoma, is negative for TdT. Translocation of *C-MYC,* located on chromosome 8, is almost always present and is detected by FISH or cytogenetics.

Large Cell/Pleomorphic Lesions

The large cell pattern encompasses a wide variety of entities, including HL and non-Hodgkin lymphomas, germ cell tumors, melanoma, sarcomas, and some carcinomas, as summarized in Table 6. In children, this category also includes granular cell tumors, tumors with gangliocytic differentiation, and lesions with multinucleated giant cells (eg, granulomatous inflammation, pilomatricoma). The morphology of these lesions is similar to that noted in adults. However, ganglion cells are infrequently encountered in adult tumors, but are more likely to be observed in children, given the higher incidence of sympathetic nervous system tumors. Ganglion cells appear as large cells with abundant cytoplasm with variably conspicuous darkly stained Nissl substance, and large round nuclei with prominent nucleoli. In contrast to melanoma, binucleation and intranuclear cytoplasmic inclusions are not common and the ganglion cells are negative for HMB-45, MelanA, and microphthalmia-associated transcription factor. The identification of ganglion cells is important because ganglioneuromas and intermixed ganglioneuroblastomas generally have a better prognosis than neuroblastoma. However, these lesions are typically excised for

definitive classification, which depends in part on the percentage of neuroblastic cells and the histological architecture.

PITFALLS AND LIMITATIONS

The cytologic diagnosis of pediatric malignancies presents several challenges, due in part to the overlapping features of these tumors. This is particularly true for SRCTs and spindle cell tumors, in which extensive morphologic overlap exists and accurate diagnoses rely heavily on ancillary studies.[3,4,12,19,25] In addition, the cytologic features of some pediatric malignancies overlap with those of benign lesions, leading to both false-negative and false-positive diagnoses. Benign lymphoid proliferations, such as infectious mononucleosis, may be misdiagnosed as lymphoma in the absence of ancillary studies, and pilomatricomas may be confused with rhabdomyosarcomas. LCH may mimic granulomatous inflammation, and reactive or other benign myofibroblastic and fibroblastic proliferations may be confused with low-grade sarcomas.[19] Other factors that impact the ability to make a definitive diagnosis include sampling error, heterogeneous tumor populations, predominant obscuring necrosis or cyst contents, and equivocal or problematic IHC results.[4] A further limitation of FNAB is that, even with an adequate sample, the precise classification of a malignancy within a diagnostic category is not always possible. Examples include the distinction between embryonal and alveolar rhabdomyosarcomas in the absence of a translocation, and differentiation between favorable and unfavorable histology neuroblastomas based on the mitotic karyorrhectic index. When treatment differs based on the precise classification of a tumor, as in these examples, a subsequent biopsy is required for further subtyping.

Conclusions

In the United States, pathologists and pediatric oncologists have been slow to embrace the use of cytopathology for the evaluation of suspected malignancies in children and adolescents, despite evidence that this modality is highly sensitive and specific, accurate, less expensive, and less invasive than either core needle or open biopsy. The widespread influence of COG protocols, which are based on histological findings, is a major deterrent to the use of FNAB, but pathologists also play a role in the underuse of this modality. Pediatric pathologists are familiar with the histologic features of malignancies arising in children and adolescents, but the majority do not perform or interpret FNABs. Conversely, cytopathologists routinely evaluate FNABs, but may rarely encounter pediatric malignancies. As a consequence, apprehension exists among a cadre of pediatric pathologists and cytopathologists regarding the diagnosis of these lesions in cytologic specimens. Thus, advocates of FNAB for the evaluation of suspected malignancies in children and adolescents often encounter resistance from clinicians, pathologists, or both. In contrast, in resource-limited regions, cytopathology is more widely accepted as a valuable diagnostic modality for pediatric malignancies. Use of a pattern-based approach, such as that described in the current review and used previously in the literature,[19] can be helpful in determining a differential diagnosis.

Over the past several decades, the incidence of cancer in children and adolescents has increased; however, the survival rate has also increased in developed countries due to improvements in diagnosis and treatment. These trends are expected to continue, with a consequent increase in the number of pediatric malignancies encountered by pathologists. The increasing use of minimally invasive diagnostic modalities and the availability of techniques that allow for the detection of important diagnostic, prognostic, and therapeutic markers with small amounts of tissue provide an opportunity to reevaluate the role of FNAB in the diagnosis of these tumors.[18] In an era of decreasing reimbursements and rising pressures to contain the costs of health care, FNAB offers a highly sensitive, specific, and accurate alternative to more costly and invasive biopsies.

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REFERENCES

1. Steliarova-Foucher E, Stiller C, Lacour B, Kaatsch P. International Classification of Childhood Cancer, third edition. *Cancer*. 2005; 103:1457-1467.
2. Sullivan R, Kowalczyk JR, Agarwal B, et al. New policies to address the global burden of childhood cancers. *Lancet Oncol*. 2013;14:e125-e135.
3. Drut R, Drut RM, Pollono D, et al. Fine-needle aspiration biopsy in pediatric oncology patients: a review of experience with 829 patients (899 biopsies). *J Pediatr Hematol Oncol*. 2005;27:370-376.

4. Razack R, Michelow P, Leiman G, et al. An interinstitutional review of the value of FNAB in pediatric oncology in resource-limited countries. *Diagn Cytopathol*. 2012;40:770-776.
5. Steliarova-Foucher E, Stiller C, Kaatsch P, et al. Geographical patterns and time trends of cancer incidence and survival among children and adolescents in Europe since the 1970s (the ACCISproject): an epidemiological study. *Lancet*. 2004;364:2097-2105.
6. Pritchard-Jones K, Kaatsch P, Steliarova-Foucher E, Stiller CA, Coebergh JW. Cancer in children and adolescents in Europe: developments over 20 years and future challenges. *Eur J Cancer*. 2006;42:2183-2190.
7. Brenner H. Up-to-date survival curves of children with cancer by period analysis. *Br J Cancer*. 2003;88:1693-1697.
8. Magrath I, Steliarova-Foucher E, Epelman S, et al. Pediatric cancer in low-income and middle-income countries. *Lancet Oncol*. 2013; 14:e104-e116.
9. Ko EY, Ritchey ML. Current management of Wilms' tumor in children. *J Pediatr Urol*. 2009;5:56-65.
10. Fernandez-Pineda I, Cabello R, Garcia-Canton JA, et al. Fine-needle aspiration cytopathology in the diagnosis of Wilms; tumor. *Clin Transl Oncol*. 2011;13:809-811.
11. Anne S, Teot LA, Mandell DL. Fine needle aspiration biopsy: role in diagnosis of pediatric head and neck masses. *Int J Pediatr Otorhinolaryngol*. 2008;72:1547-1553.
12. Cohen MB, Bottles K, Ablin AR, Miller TR. The use of fine-needle aspiration biopsy in children. *West J Med*. 1989;150:665-667.
13. Silverman JF, Gurley AM, Holbrook CT, Joshi VV. Pediatric fine-needle aspiration biopsy. *Am J Clin Pathol*. 1991;95:653-659.
14. Ponder TB, Smith D, Ramzy I. Lymphadenopathy in children and adolescents: role of fine-needle aspiration in management. *Cancer Detect Prev*. 2000;24:228-233.
15. Howell LP. Changing role of fine-needle aspiration on the evaluation of pediatric masses. *Diagn Cytopathol*. 2001;24:65-70.
16. Verdeguer A, Castel V, Torres V, et al. Fine-needle aspiration biopsy in children: experience in 70 cases. *Med Pediatr Oncol*. 1988;16:98-100.
17. O'Leary M, Krailo M, Anderson JR, Reaman GH; Children's Oncology Group. Progress in childhood cancer: 50 years of research collaboration, a report from the Children's Oncology Group. *Semin Oncol*. 2008;35:484-493.
18. Kanagal-Shamanna R, Portier BP, Singh RR, et al. Next-generation sequencing-based multi-gene mutation profiling of solid tumors using fine needle aspiration samples: promises and challenges for routine clinical diagnostics [published online ahead of print August 2, 2013]. *Mod Pathol*. doi: 10.1038/modpathol. 2013.122.
19. Howell LP, Russell LA, Howard PH, Teplitz RL. The cytology of pediatric masses: a differential diagnostic approach. *Diagn Cytopathol*. 1992;8:107-115.
20. Abla O, Egeler RM, Weitzman S. Langerhans cell histiocytosis: current concepts and treatments. *Cancer Treat Rev*. 2010;36:354-359.
21. Boonyaarunnate T, Olson MT, Bishop JA, Yang GC, Ali SZ. Cribriform morular variant of papillary thyroid carcinoma: clinical and cytomorphological features on fine-needle aspiration. *Acta Cytol*. 2013;57:127-133.
22. Wohllk N, Schweizer H, Erlic Z, et al. Multiple endocrine neoplasia type 2. *Best Pract Res Clin Endocrinol Metab*. 2010;24:371-387.
23. Wakely PE Jr, Silverman JF, Geisinger KR, Frable WJ. Fine needle aspiration biopsy cytology of hepatoblastoma. *Mod Pathol*. 1990;3: 688-693.
24. Iyer VK, Kapila K, Agarwala S, Verma K. Fine needle aspiration cytology of hepatoblastoma. Recognition of subtypes on cytomorphology. *Acta Cytol*. 2005;49:355-364.
25. Viswanathan S, George S, Ramadwar M, Medhi S, Arora B, Kurkure P. Evaluation of pediatric abdominal masses by fine-needle aspiration cytology: a clinicoradiologic approach. *Diagn Cytopathol*. 2010;38:15-27.
26. Barwad A, Gupta N, Gupta K, et al. Hepatoblastoma-an attempt of histologic subtyping on fine-needle aspiration material. *Diagn Cytopathol*. 2013;41:95-101.
27. Klatte T, Streubel B, Wrba F, et al. Renal cell carcinoma associated with transcription factor E3 expression and Xp11.2 translocation incidence characteristics and prognosis. *Am J Clin Pathol*. 2012; 137:761-768.
28. Srigley JR, Delahunt B. Uncommon and recently described renal carcinomas. *Mod Pathol*. 2009;22(suppl 2):S2-S23.