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CYTOPATHOLOGY

Atypical Teratoid/Rhabdoid Tumor of the Brain

Cytopathologic Characteristics and Differential Diagnosis

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BACKGROUND. Atypical teratoid/rhabdoid tumor (AT/RT) is a highly aggressive neoplasm with a unique cytogenetic profile. Although the clinicopathologic and radiologic features of AT/RT have been described previously, to the authors' knowledge the cytomorphologic profile of this tumor has not been studied well.

METHODS. Nine samples of AT/RT from 8 patients were analyzed from the pathology files of 2 large institutions in a 10-year period (1993–2002). Material consisted of slides made from scraping and smearing (SS) or squash preparation (SP) of the tissue cores (six slides), fine-needle aspiration (FNA) (two slides), and cerebrospinal fluid (one slide). Smears were stained with Diff-Quik, Papanicolaou, and hematoxylin and eosin stains.

RESULTS. There were 4 males and 4 females who ranged in age from 1–16 years (mean age, 7.1 years). Cytomorphologic features consisted of hypercellularity (eight of eight tumors); predominantly large tissue fragments with tumor cells surrounding proliferating capillaries depicting a "papillary-like" appearance (five of eight tumors); large, round, "plasmacytoid" cells and characteristic "rhabdoid" cells (i.e., intermediate-sized cells with granular to fibrillary, brightly eosinophilic cytoplasm with or without globoid "inclusions"; large, eccentrically located, round-to-reniform nuclei with single prominent nucleoli; eight of eight tumors); small, round, primitive "neuronal-appearing" cells with a high nuclear to cytoplasmic ratio (five of eight patients); and bizarre, multinucleated giant cells (two of eight tumors). Also seen were numerous apoptotic bodies, mitoses, and significant necrosis (seven of eight tumors), and prominent dystrophic calcification (four of eight tumors).

CONCLUSIONS. AT/RT is extremely rare. Cytologic examination by SS, SP, or FNA offers a useful alternative to frozen section during intraoperative consultation. Cytomorphologic features are unique and lead to an accurate diagnosis in the right clinicoradiologic context. The differential diagnosis includes medulloblastoma (in cerebellar tumors), primitive neuroectodermal tumor (in suprasellar tumors), choroid plexus carcinoma, and malignant glioma. ***Cancer (Cancer Cytopathol)* 2005;105:65–70.** © *2005 American Cancer Society.*

KEYWORDS: atypical teratoid rhabdoid tumor, primitive neuroectodermal tumor, medulloblastoma, brain, cytopathology.

Atypical teratoid/rhabdoid tumor (AT/RT) is a highly aggressive neoplasm with a unique cytogenetic profile[1–3] and a distinctive population of large, "rhabdoid" cells seen in the central nervous system (CNS) of children (usually age $<$ 2 years).[4–7] AT/RTs have morphologic features of both medulloblastoma (MB) and primitive neuroectodermal tumor (PNET) with epithelial, primitive neuroepithelial, and mesenchymal differentiation. They can also closely resemble choroid plexus carcinoma. The large rhabdoid cells seen in AT/RT were described first in association with a unique pediatric

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kidney neoplasm termed rhabdoid tumor of infancy.[5,8] The rhabdoid phenotype subsequently was noted in extrarenal sites as well, including the CNS. Rorke et al.[9] at The Children's Hospital of Philadelphia in 1987, coined the term "AT/RT" to describe these biologically unique neoplasms with aggressive behavior and characteristic morphologic features.[5,7–10]

Ultrastructurally, the rhabdoid cells, which can be prominent but often not dominant, show intracytoplasmic, paranuclear whorls of intermediate filaments. Molecular/cytogenetic analyses frequently have shown partial or complete deletions of chromosome 22 in both renal and extrarenal rhabdoid tumors, confirming their common origin.[11,12] Mutations/deletions in the *INI1* gene appear to be responsible for this lesion.[12] Further evidence of a common origin of AT/RT and renal rhabdoid neoplasms is provided by the simultaneous presence of both neoplasms in the same patient.[5] A study by Weeks et al. of renal rhabdoid neoplasms (n = 111) showed that 13.5% of these patients also had concurrent CNS AT/RT.[13]

Although the clinicopathologic and radiologic features of AT/RT have been described previously, to our knowledge the cytomorphologic profile of this tumor has not been studied, with only rare case reports appearing in the cytopathology literature.[14,15] In this report, we describe the clinicoradiologic and cytomorphologic features of nine cases of AT/RT, with emphasis on their unique morphologic appearance; then, the differential diagnosis is discussed based on the anatomic location and the patient's age.

MATERIALS AND METHODS

A retrospective search of the pathology files at The Johns Hopkins Hospital and University of Minnesota (between 1993–2002) revealed nine cases of AT/RT from eight patients with cytologic material. A comprehensive cytomorphologic analysis was then undertaken with histopathologic and clinicoradiologic correlation.

Material consisted of slides made from scraping and smearing (SS) or squash preparation (SP) of the tissue cores (six slides) performed at the time of intraoperative consultation, fine-needle aspirates (FNA) (two slides), and cerebrospinal fluid (CSF) (one slide). FNA was performed by a team comprised of a neurosurgeon and a neuroradiologist assisted by a cytopathologist. A dedicated neuroradiology suite was utilized with computerized tomography scanning under general anesthesia. The aspirated material was used to prepare smears that were air-dried and stained with Diff-Quik for an immediate on-site evaluation or wet-fixed in ethanol for subsequent Papanicolaou staining.

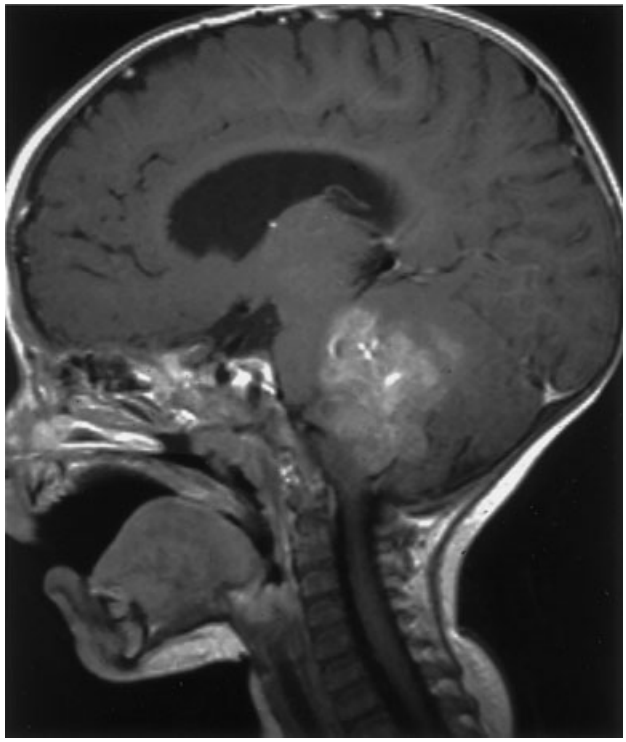

FIGURE 1. Atypical teratoid/rhabdoid tumor. This sagittal-plane magnetic resonance image of the brain shows a large, bulky, posterior fossa mass.

Four-micron cell block sections were prepared from needle rinses and stained with hematoxylin and eosin (H & E). For SS, the cores were scraped gently with a scalpel blade, and the resulting material was smeared on a glass slide. For SP, minute fragments of tissue were separated from the main core and then gently squashed between two glass slides. Both SS and SP slides were wet-fixed immediately in 95% ethanol and stained with H & E. Finally, correlation with the radiologic findings and histopathologic follow-up was performed, whenever available.

RESULTS

Patient Demographics, Clinical Data, and Radiologic Data

The patient population consisted of 4 males and 4 females (male-to-female ratio, 1:1) with an age range of 1–16 years (mean age, 7.1 years). The radiologic appearance of tumors was highly variable, with some tumors presenting as contrast-enhancing, partially cystic/hemorrhagic masses, and with other tumors that were predominantly solid (Fig. 1). Tumor size ranged from 2.0–6.7 cm (mean size, 3.7 cm). Anatomic locations included the cerebellum, third ventricle, pineal region, and frontal lobes (Tables 1 and 2).

TABLE 1
Demographic Data and Radiologic Findings

Case no.	Age (yrs)	Gender	Anatomic location and radiologic findings
1	5	Male	Suprasellar mass
2	5	Male	Left frontal lobe, intraventricular mass
3, 4	16	Female	Pineal, midbrain, ill-defined mass
5	5	Female	Not available
6	1	Male	Area of the foramen of Luschka extending to the brainstem and the middle cerebellar peduncle, cystic and solid mass
7	16	Female	Cerebellar vermis and right frontal lobe, partially cystic mass
8	5	Male	Left temporoparietal, partially cystic mass with midline shift
9	3	Female	Right temporal lobe, partially cystic and solid hemorrhagic mass (6.0 × 5.0 cm) associated with a 4-mm, right-to-left midline shift

TABLE 2
Salient Clinical Presentation

Recurrent headaches
Seizures
Nausea/emesis
Progressive motor weakness
Difficulty swallowing

TABLE 3
Cytomorphologic Characteristics

Cytomorphologic features	Incidence
Hypercellularity with tissue fragments and single cells	100%
Perivascular aggregation around branching vessels or "papillary-like"	62%
Large, round, and pleomorphic "plasmacytoid" cells	100%
"Rhabdoid" cells with brightly eosinophilic cytoplasm; large, eccentrically-placed nuclei; and single, prominent nucleoli with or without fibrillary globoid inclusion	100%
Predominance of high N/C ratio, primitive "neuronal-appearing" cells	62%
Pleomorphic, multinucleated giant cells	25%
Apoptosis, mitoses, necrosis	87%
Dystrophic calcifications	50%

N/C ratio: nuclear to cytoplasmic ratio.

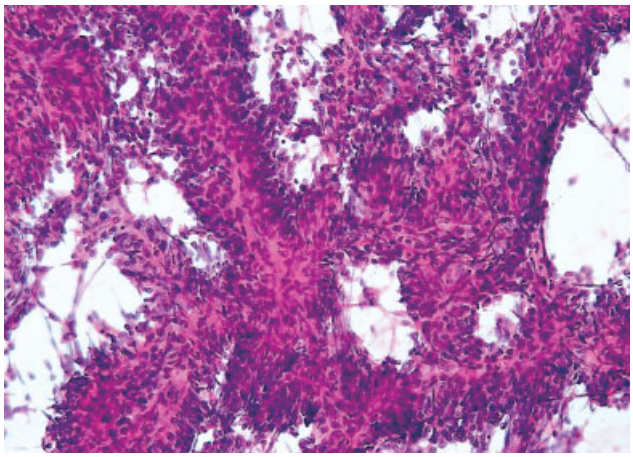

FIGURE 2. Atypical teratoid/rhabdoid tumor (squash preparation). This hypercellular smear shows fragments of neoplastic cells arranged in a branching, "papillary-like" architecture (H & E, original magnification × 100).

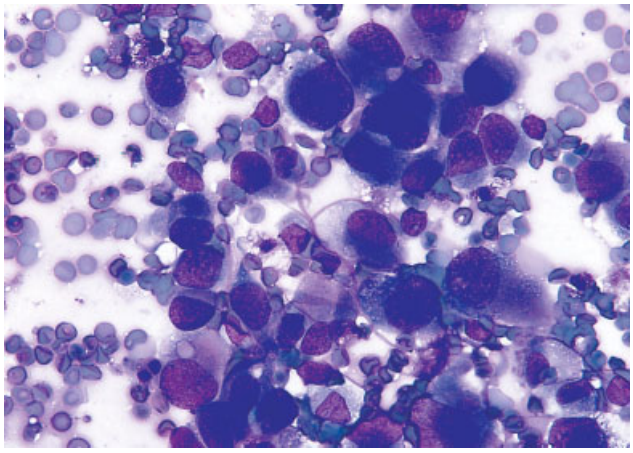

FIGURE 3. Atypical teratoid/rhabdoid tumor (squash preparation). Large, pleomorphic, "plasmacytoid," and polygonal cells are seen with eccentrically placed nuclei and dense amphophilic cytoplasm (Diff Quik, original magnification × 400).

Cytomorphologic Features

Except for a solitary case of AT/RT observed in the CSF, all other tumors showed extreme hypercellularity (8 of 8 tumors; 100%) (Table 3). The predominant tumor architecture was large tissue fragments with neoplastic cells surrounding proliferating capillaries or avascular cores, thus depicting a "papillary-like" appearance (5 of 8 tumors; 62%) (Fig. 2). Higher magnification showed large, pleomorphic, round "plasmacytoid" cells with dense amphophilic cytoplasm and no nucleoli (Fig. 3) or the characteristic, so-called "rhabdoid" cells (i.e., intermediate-sized cells with granular-to-fibrillary, brightly eosinophilic cytoplasm with or without globoid "inclusions"; and large, eccentrically located, round-to-reniform nuclei with single, prominent nucleoli; 8 of 8 tumors; 100%) (Fig. 4). Also present were small, round, primitive, "neuronal-appearing" cells that were characterized by a high nuclear/cytoplasmic ratio, speckled chromatin, and small nucleoli (5 of 8 tumors; 62%). These cells often were associated with fine-branching capillary vessels (Figs. 5 and 6). Few tumors showed significant cellular pleomorphism with bizarre, multinucleated giant cells (2 of 8 tumors; 25%) (Fig. 7). Also seen were numerous apoptotic bodies, mitoses, and areas of necrosis (7 of 8 tumors; 87%) and prominent dystrophic calcifica-

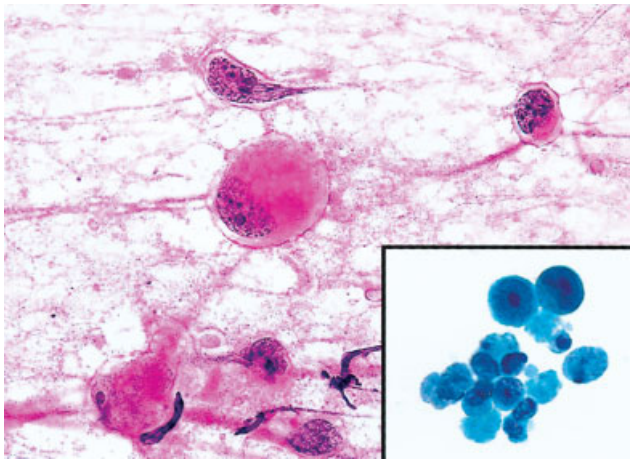

FIGURE 4. Atypical teratoid/rhabdoid tumor (fine-needle aspirate). The characteristic "rhabdoid" cells are seen with eccentrically placed nucleus, large nucleolus, and eosinophilic-globoid cytoplasmic inclusion. *Inset*: malignant cells with a high nuclear/cytoplasmic ratio and single, prominent nucleoli. Apoptotic bodies also are present (H & E and Papanicolaou stain, original magnification × 600).

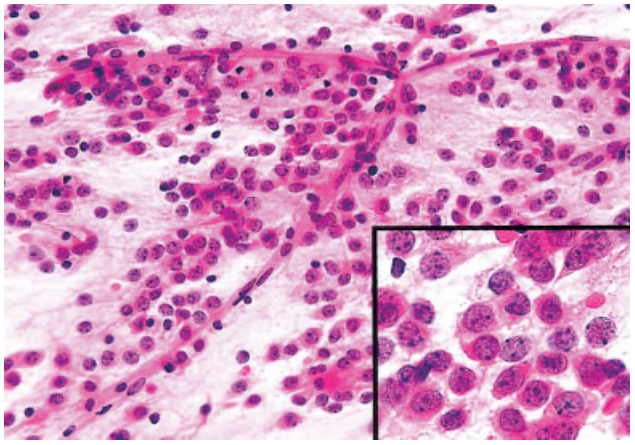

FIGURE 5. Atypical teratoid/rhabdoid tumor (scrape and smear preparation). Small, monomorphic, "neuronal-type" cells are seen. Note the fine-branching capillary vessels and the lack of fibrillary background (H & E, original magnification × 200; *Inset*: × 400).

tion (4 of 8 tumors; 50%). Cellular rosettes, epithelial or germ cells, and neoplastic mesenchyme were not present. None of the tumors demonstrated cellular/nuclear molding, cytoplasmic vacuolization, spindled cells, or fibrillary smear background. No significant cytomorphologic differences were observed between SS, SP, or FNA slides. A CSF examination revealed only single neoplastic cells that had a "rhabdoid" appearance.

Histopathologic follow-up showed densely cellular zones comprised of intermediate-sized, rhabdoid-type cells with eccentrically placed nuclei and brightly

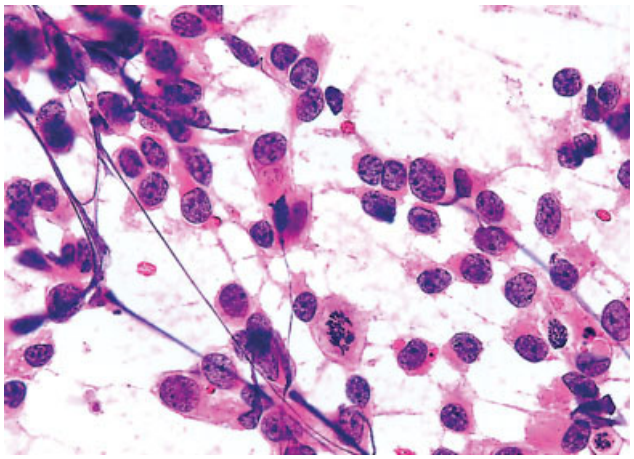

FIGURE 6. Atypical teratoid/rhabdoid tumor (squash preparation). Neoplastic cells are seen with mild anisonucleosis, mitosis, and focal nuclear crush artifact. Nuclei lack prominent nucleoli and rhabdoid phenotype (H & E, original magnification × 400).

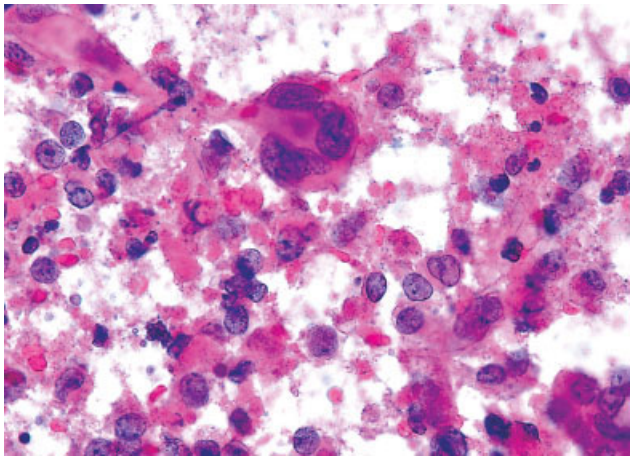

FIGURE 7. Atypical teratoid/rhabdoid tumor (squash preparation). Pleomorphic, multinucleated giant cells are associated with necrosis and granular-appearing debris (H & E, original magnification × 400).

eosinophilic cytoplasm (Fig. 8). A few tumors showed a predominance of smaller, "neuronal-type" cells with only rare larger cells that had a rhabdoid phenotype. Areas of necrosis and calcification were present in the majority of the tumors.

DISCUSSION

AT/RTs of the CNS are aggressive childhood neoplasms that are located most commonly in the posterior intracranial fossa (approximately two-thirds of cases).[3] These tumors are rare, with an estimated incidence of 2–3% of primary CNS tumors in children age ≤ 18 years, and there is a slight male predominance (1.6:1.0).[1,5,6,8,16] Most patients are age < 2

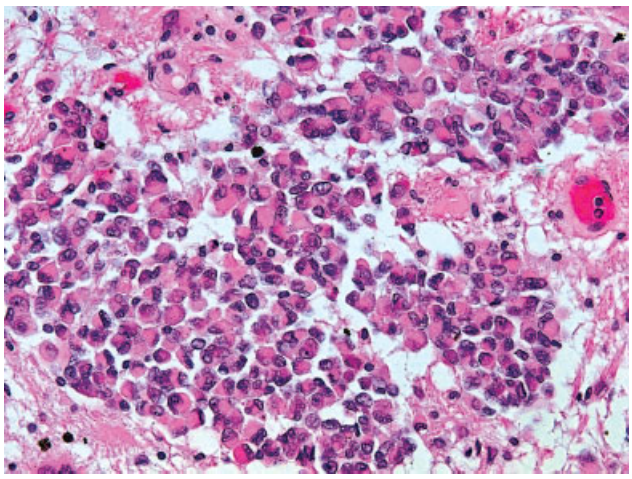

FIGURE 8. Atypical teratoid/rhabdoid tumor (histologic section). Nests of neoplastic cells are seen with the characteristic "rhabdoid" phenotype arranged in an alveolar fashion (H & E, original magnification × 200).

years[17]; however, some older children and even adults with AT/RT have been described.[5,15] In the current study, there was a wide range of ages (1–16 years) with a mean age of 7.1 years.

Imaging studies, particularly the use of magnetic resonance imaging, are useful initial diagnostic modalities. Most of the lesions are bulky, contrast enhancing with hemorrhage and necrosis.[18] The histopathologic spectrum of AT/RT is broad, ranging from predominantly "small cell" with primitive morphology to tumors with large rhabdoid cells. In addition, some AT/RTs may have mesenchymal and epithelial components. Because of this morphologic variability, AT/RTs often have been misclassified.[3–5,8,17,19]

Cytomorphologically, the smears are hypercellular with primitive-appearing, neoplastic cells admixed with intermediate-sized, rhabdoid cells in varying proportions. The rhabdoid cells have prominent nucleoli and conspicuous, spherical, cytoplasmic inclusions. Mitoses, necrosis, and dystrophic calcification also may be present. Perivascular, pseudopapillary structures also have been described.[8] Lu et al. described CSF findings in a girl age 2 years with AT/RT. In their study, the most consistent cytologic findings were the large size of the tumor cells, eccentricity of the nuclei, and prominent nucleoli.[14] The immunophenotypic profile of AT/RT is broad, showing variable reactivity with epithelial membrane antigen, glial fibrillary acidic protein, cytokeratins, and (less frequently) with actin, neurofilaments, and chromogranin.[9] A recently described antibody to *INI1* has been used in the differential diagnosis.[12]

The differential diagnosis includes MB/PNET, choroid plexus carcinoma, gemistocytic astrocytoma, oligodendroglioma, and non-Hodgkin lymphoma (NHL). It is important to consider the diagnosis of AT/RT in patients age < 1 year who have specimens that demonstrate cytomorphologic features of MB or PNET.[8] AT/RT may show a cytomorphology of predominantly primitive-looking neuronal cells mimicking MB or PNET. In general, MB/PNET will show hyperchromatic nuclei, often with significant anaplasia (nuclear enlargement, nuclear molding, etc.) in addition to focal rosette formations. Finding larger cells with eccentric nuclei or rhabdoid cells argues against a diagnosis of MB/PNET and should be considered a characteristic feature of AT/RT.

Gliomas show significant pleomorphism with a characteristic fibrillary background. The neoplastic cells are dispersed singly and are not associated with perivascular proliferation or papillary-like arrangements as are seen in AT/RT. Care must be taken, however, not to confuse gemistocytes in a glioma with the rhabdoid cells of an AT/RT. Gemistocytes are significantly larger cells with abundant, glassy-looking cytoplasm and often are binucleated. Oligodendroglioma shows small, uniform cells, often with fragile cytoplasm, leading to a large population of naked, "lymphocyte-like" nuclei. Microgemistocytes occasionally may be seen in these neoplasms. NHL will have cells with scant basophilic cytoplasm or naked nuclei, often with irregular-shaped nuclei and prominent nucleoli. Lymphoglandular bodies also may be helpful, as well as a large population of crushed naked nuclei.

One clinicopathologic study characterized 55 patients with AT/RT.[4] The tumors occurred primarily in children age < 2 years with a mean age of 17 months. The most common locations were the posterior fossa (n = 36 tumors) and the supratentorial compartment (n = 17 tumors).[4] The tumors were immunopositive for vimentin, glial fibrillary acidic protein, epithelial membrane antigen, cytokeratins, synaptophysin, chromogranin, and smooth muscle actin.[4] A large earlier series described the clinical and pathologic features in 52 infants and children.[9] Those investigators noted that AT/RT may be misdiagnosed as PNET, because 70% of AT/RTs have areas that are indistinguishable from classic PNET. The tumors may be composed entirely (13%) or partially (77%) of rhabdoid cells.

Cytogenetic and other molecular studies have determined that AT/RT from the CNS is related closely to renal and other extrarenal rhabdoid tumors. Deletions and mutations of the *hSNF5/INI1/SMARCB1* locus in chromosome band 22q11.2 has been documented in rhabdoid tumors of the kidney, CNS (AT/RT), and

other extrarenal sites.[6,11,12,20] The exact function of *INI1* in rhabdoid tumors is unknown; however, the leading hypothesis is that *INI1* may function as a tumor-suppressor gene affecting the transcription of cellular genes. The immunohistochemical reagent for *INI1* fails to stain the nuclei of tumor cells with *INI1* mutations but does stain the cells of normal or reactive tissue as well as those of other neoplasms in the differential diagnosis.[12]

In the current study, we described the cytomorphologic features of eight patients with AT/RT from a pediatric population. When confronted with AT/RT, the differential diagnosis for the cytopathologist may be challenging due to overlapping morphologic features and a variable presence of primitive, mesenchymal, and/or epithelial components. The presence of large rhabdoid cells may be helpful, but such cells are not always present and the immunophenotypic profile of AT/RT is highly variable. These tumors may be misdiagnosed as MB or PNET. Other entities in the differential diagnosis include choroid plexus carcinoma or germ cell tumors. Molecular genetic analysis of the *INI1* gene may be useful in confirming the diagnosis of AT/RT.[4,6]

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