Letter to the Editor

A pleomorphic xanthoastrocytoma highlighting the morphological heterogeneity of this uncommon tumor

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To the Editor:
Pleomorphic xanthoastrocytomas (PXAs) are rare, astrocytic gliomas that account for less than 0.3% of all primary central nervous system (CNS) tumors; they have a predilection for supratentorial locations involving the leptomeninges and cerebral cortex (1). They can occur in any region of the CNS but are most frequently found in temporal lobes. Clinical presentation of PXAs is most often characterized by children or young adults presenting with a new onset of epileptic seizures (2). In 2021, the World Health Organization (WHO) defined PXAs as astrocytomas with large pleomorphic (frequently multinucleated) cells, spindle cells, and lipidized cells, often with numerous eosinophilic granular bodies, pericellular reticulin deposition, BRAF p.V600E mutations, and homozygous cyclin-dependent kinase inhibitor 2A/2B (CDKN2A/2B) deletions (2). They are further categorized as CNS-WHO Grade 2 tumors if their mitotic activity is less than 5 mitoses per 10 high-powered fields (HPFs). A CNS-WHO Grade 3 is given to these tumors when there are 5 or more mitoses per 10 HPFs. Contrary to what their pleomorphic histology may suggest, PXAs usually behave in a benign fashion. Total resection has been found to be the most significant prognostic factor linked to recurrence with increased mitotic activity indicating poorer outcomes (2).

We report the case of a 43-year-old woman with a past medical history significant for type 2 diabetes mellitus who presented with worsening, progressive neurological symptoms of paresthesias and pain in the hands and bilateral lower extremities (right > left) that had initially started in 2018. Clinical concern was raised for a possible diabetes-associated peripheral neuropathy. Skin biopsies were taken from the right distal leg, right distal thigh, and right proximal thigh; all 3 showed similar findings with no histopathological evidence of a small fiber sensory neuropathy. An electromyography/nerve conduction study of the lower extremities showed no abnormalities.

After the inconclusive initial clinical work-up, a brain magnetic resonance imaging (MRI) was pursued. She was initially followed with interval imaging, but a 3-month follow-up brain MRI demonstrated a well-circumscribed, enhancing, cortically based mass (1.0 cm × 0.9 cm) in the left parietal lobe (Fig. 1). Intraoperatively, a well-circumscribed, firm, purple-colored mass centered in a left parietal sulcus near the cortical surface was found and a gross total resection of the mass was achieved. The patient experienced post-operative aphasia that progressively improved by work with a speech therapist. Her initial presenting symptomatology of paresthesia and pain in the extremities has since resolved.

Histological analysis revealed a non-infiltrating, well-circumscribed glioma. The tumor cells displayed a diverse array of intermixed cytological features, including astrocytic, spindle cell, and epithelioid morphologies. The cellularity was high. The tumor cells were arranged in uniform lobules separated by intervening dark-pink, collagenous fibers admixed with lymphocytic inflammatory infiltrates (Fig. 2A, B). The mitotic index was up to 1 mitosis per 10 high-power field (HPF). Tumor necrosis and microvascular proliferation were not identified.

IHC stains subsequently showed the following: GFAP stain displayed strong, diffuse positivity in tumor cells (Fig. 2C). IDH1-R132H stain was negative in tumor cells. The ATRX stain demonstrated retained nuclear expression in tumor cells. CD34 stain highlighted focal cytoplasmatic staining in tumor cells while also highlighting unremarkable endothelial cells associated with vessels (Fig. 2D). Anti-synaptophysin and -chromogranin A both demonstrated weak focal positivity (Fig. 2E, F). Neurofilament stain highlighted the non-infiltrating, well-circumscribed nature of the tumor by demonstrating that there were no...
intervening axons surrounded by tumor cells (Fig. 2G). A reticulin stain demonstrated increased reticulin fiber deposition encircling tumor cell aggregates that resulted in uniform lobular architectural structures (Fig. 2H). Pertinent negative stains performed to rule-out metastatic carcinoma and melanoma included immunostains for CAM5.2, HMB-45, and Melan-A.

Epidermal growth factor (EGFR) amplification studies by fluorescence in situ hybridization (FISH) analysis showed the tumor to be EGFR non-amplified. Droplet digital polymerase chain reaction (ddPCR) was performed to assess for the DNA reparative enzyme O(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation status; MGMT promoter methylation was not demonstrated in the tumor cells. BRAF codon 600 mutation analysis via pyrosequencing detected a BRAF c.1799T>A (p.V600E) variant.

Whole genome methylation profiling was performed at the National Institute of Health (NIH) on the tumor tissue block for further subclassification. The composite methylation profile utilized the Heidelberg classifier versions 11b6 and 12b6, as well as the National Cancer Institute/Bethesda classifier. The presence of a CDKN2A deep deletion was identified. The consensus methylation profiling class using all three of the aforementioned classifiers indicated a PXA with a high confidence methylation class score (>0.9). The distinction between a CNS-WHO Grade 2 or 3 PXA is currently not possible by NIH methylation profiling standards. Thus, after considering the various histopathological features, molecular results, and methylation profile classification, a final integrated diagnosis was rendered and reported as a PXA, CNS-WHO Grade 2.

In summary, the tumor tissue sections for this case demonstrated a non-infiltrating, well-circumscribed low-grade glioma arranged in uniform lobules with lymphocytic infiltrates interspersed between the collagenous septa of the lobules. The IHC work-up further characterized the tumor cells as having strong GFAP positivity, weakly positive CD34 stromal staining, focal weak positivity in the synaptophysin and chromogranin A (highlighting a neuronal component), and a neurofilament stain which failed to highlight axons surrounded by tumor cells indicating the non-infiltrative nature of the tumor. The reticulin stain further emphasized the rich reticulin network surrounding the aggregated tumor cells displaying the lobular architectural pattern. Genetic testing of the neoplasm revealed a BRAF V600E mutation and a CDKN2A homozygous deletion. NIH methylation-based tumor profiling further subclassified the neoplasm as a PXA with a final integrated diagnosis of a PXA, CNS-WHO grade 2.

Histopathology of PXAs was first described in 3 almost identical cases consisting of large, pleomorphic cells with numerous tumor cells possessing vacuolated cytoplasm comprised of lipids (3). Since this initial discovery, the classical morphology of PXAs has included: pleomorphic tumor cells with bizarre cytological features (i.e. multi-nucleated giant cells), spindle cell components with a fascicular or storiform pattern, foamy lipid-laden xanthomatous cells, and eosinophilic granular bodies (2). Unique histopathology with an abundant clear cell component and focal papillary appearance has also been reported (4). The varying morphologies comprising PXAs further highlights the pleomorphic nature of these tumors.

A noteworthy seminal study by Giannini et al. comprehensively examined and documented the frequency of specific histological features involving 71 cases of PXAs (5). The reported histological features with associated frequencies included: multinucleated giant cells (92%), xanthomatous cells (66%), granular bodies (93%), Rosenthal fibers (27%), nuclear inclusions (87%), reticulin (52%), lymphocyte collections (83%), calcifications (18%), and GFAP reactivity (41%) (5). For this PXA case, there were no multinucleated giant cells, xanthomatous cells, eosinophilic granular bodies, Rosenthal fibers, nuclear inclusions, or calcifications identified. However, the tumor did show intra-septal lymphocytic collections, reticulin deposition around lobules of tumor cells, and GFAP immunoreactivity. This emphasizes the absence of the most common and classic histological attributes for PXAs in this case.

The most common mutation associated with PXAs occurs in the BRAF gene which codes for a highly oncogenic serine/threonine protein kinase enzyme that is involved with the mitogen-activated protein kinase (MAPK) cell signaling pathway (6). It has been reported that BRAF mutations are found in approximately 70% of PXAs and include BRAF p.V600E mutations, in-frame BRAF exon deletions, and less frequently BRAF fusions (6). Additionally, PXAs are characterized by a homozygous deletion at 9p21.3 encompassing CDKN2A/B which encodes the p16INK4a tumor suppressor, which is a central regulator of cell cycle progression from the G1 phase to the S phase (7). Inactivation of the p16 protein is also a
frequent finding in a vast assortment of tumors, including a significant proportion of IDH-mutant infiltrating gliomas.

In 1979, Kepes et al (8) put forth the hypothesis that the precursor cells for PXAs are the subpial astrocytes, which are specialized astrocytes found deep to the pia mater. This was further supported by evidence observed on electron microscopy where many of the PXA tumor cells were enveloped by a layer of basal laminae (8). Since subpial astrocytes are known to have this layer of basal lamina associated with them, it is highly likely that they are the origin cells of PXAs (8). Reticulin staining further helps to highlight the reticulin fibers that comprise the basal lamina component surrounding these individual tumor cells. Furthermore, this rich network of reticulin deposition bestows a distinct morphological feature to PXAs helping to distinguish them from other glial and glioneuronal entities.

In our case, the reticulin stain demonstrated strong reticulin fiber deposition around large, uniform lobules. (H) Reticulin stain demonstrates prominent deposition around tumor cell aggregates of tumor cells. Scale bars: (A) = 50 μm; (B–F) = 100 μm; (G) = 50 μm; and (H) = 100 μm.

Figure 2. Neuropathological assessment of the tumor sections. (A and B) H&E stain of tumor sections demonstrating uniform, lobular architecture. (C) Anti-GFAP shows positively stained tumor cells. (D) Anti-CD34 highlights focal cytoplasmic staining of tumor cells. (E and F) Synaptophysin and chromogranin A stains show focal weak positivity in tumor cells. (G) Neurofilament stains are negative for axons within tumor lobules. (H) Reticulin stain demonstrates prominent deposition around tumor cell aggregates of tumor cells. Scale bars: (A) = 50 μm; (B–F) = 100 μm; (G) = 50 μm; and (H) = 100 μm.
infiltration by tumor cells. While the idea of leptomeningeal involvement in this case is speculative, it could help to explain the peculiar architectural variation. This case further demonstrates the diagnostic challenges that arise when trying to differentiate PXAs from other low-grade gliomas or glioneuronal tumors. At the same time, this case further highlights the importance of the reticulin stain in the diagnostic work-up.

This case further demonstrates the morphological heterogeneity observed in PXAs while highlighting the importance of DNA methylation profiling. Given the broad spectrum of morphological presentations of PXAs, diagnostic evaluation of these tumors can be incredibly challenging. Our understanding of the molecular signatures of PXAs is of the utmost importance especially when histological analysis alone may be insufficient to diagnose this morphologically heterogeneous group of tumors. Overall, the clinical significance of this PXA displaying a broader histological spectrum than previously described has yet to be determined.

**CONFLICT OF INTEREST**
The authors have no duality or conflicts of interest to declare.

**REFERENCES**
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