FOXO1 Rearranged Alveolar Rhabdomyosarcoma with Aberrant Strong and Diffuse NKX2.2 Expression: Diagnostic Pitfall with Distinct Therapeutic Implication

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A 5-year-old male child presented with a gradually progressive mass lesion over the left shoulder region for the past 2 weeks. On local examination, there was a 4×5 cm mass over the anterior aspect of the shoulder, fixed to the deltoid muscle, as seen on the magnetic resonance imaging scan (►**Fig. 1**). The patient underwent a needle biopsy, and histopathology showed a malignant small round cell tumor (MSRCT). On the first immunohistochemistry (IHC) panel, the tumor cells were diffusely positive for NKX2.2, CD99, Desmin, Myogenin, and MyoD1, while being negative for Pancytokeratin, LCA, Synaptophysin, and NKX3.1 (►**Fig. 2**). In view of the ambiguous IHC findings, additional tests, including MUC-4 and ALK-1 (►**Fig. 3**), were performed. Both showed diffuse positive expression, and a final diagnosis of

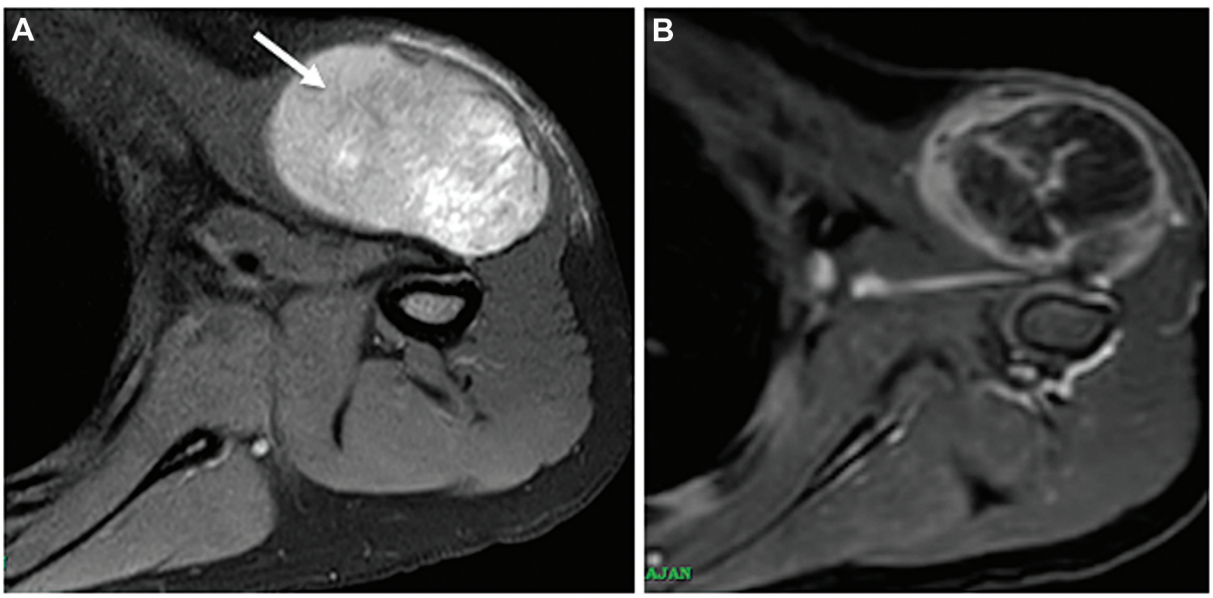

Fig. 1 T2-weighted MRI showing a hyperintense soft tissue mass in the left shoulder with heterogeneous enhancement, involving the deltoid muscle (*arrow*). No erosion of underlying bone seen.

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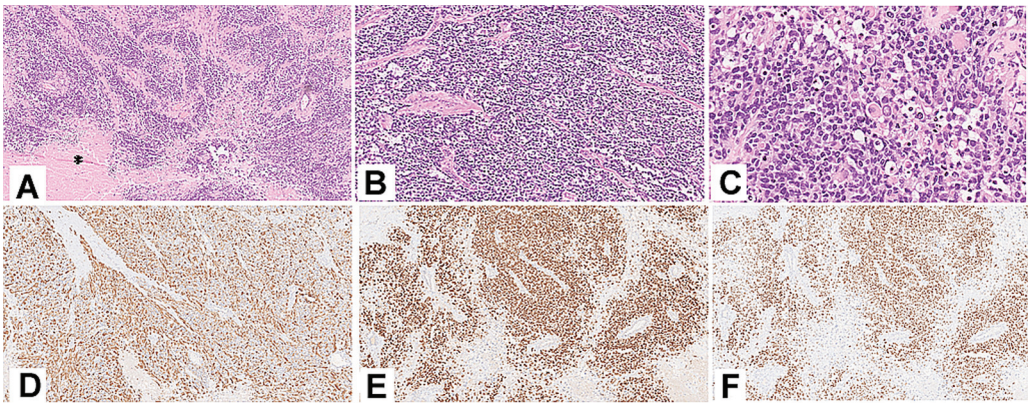

Fig. 2 (**A–C**) (10×, 20× 40×, H&E stain) Histopathology images showing a round cell tumor with extensive necrosis (*). **(D–F)** (20×, DAB stain) Immunohistochemistry images showing diffuse positivity for Desmin, Myogenin, and MyoD1, respectively.

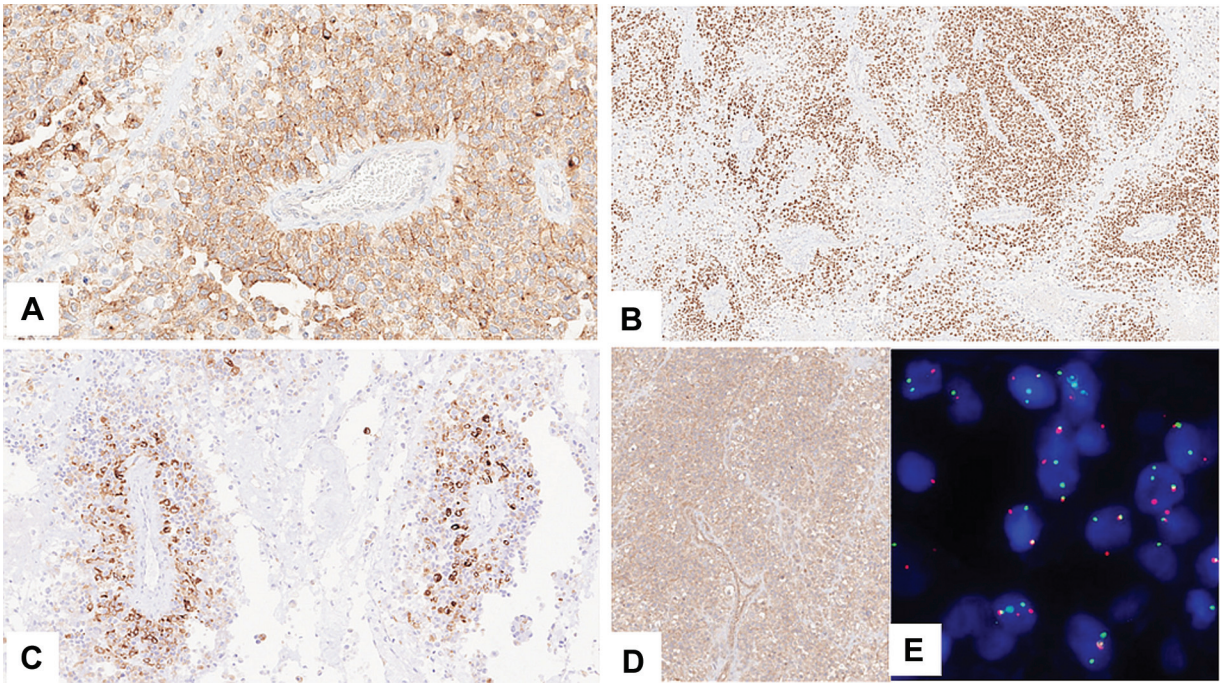

Fig. 3 (**A**) (20×, DAB stain): Tumor cells show diffuse and strong immunoreactivity for ALK-1 on immunohistochemistry. (**B**) (10×, DAB stain): immunohistochemistry images showing strong and diffuse NKX2.2 positivity and (**C**) MUC4 expression (**D**) while being negative for CD 99. (**E**) FOXO1 rearrangement seen on BA FISH.

Rhabdomyosarcoma (RMS) was rendered. Subsequently, break-apart fluorescence in situ hybridization (BA-FISH) revealed *FOXO1* gene rearrangement in 75% of the tumor cells, clinching the diagnosis of Alveolar RMS (fusion positive) with aberrant diffuse NKX2.2 immunoexpression.

The case was discussed in the multispecialty tumor board meeting and in view of no underlying bone involvement, a wide local excision was performed, followed by adjuvant chemotherapy with vincristine, actinomycin-D, cyclophosphamide (VAC) alternating with vincristine, irinotecan. After four cycles of chemotherapy, radiotherapy to the tumor bed was administered. Currently the child is receiving the remaining adjuvant chemotherapy and remains disease free.

NKX2.2 is an established IHC marker that is highly sensitive and specific for Ewing sarcoma (ES), aiding in the diagnostic workup of MSRCTs.[1,2] Alveolar RMS (ARMS) is an MSRCT with skeletal muscle differentiation, characterized by the presence of *PAX3-FOXO1* or *PAX7-FOXO1* gene fusions. Both of these entities have a similar clinical presentation, occurring in the deep soft tissues of children and young adults, but they differ widely in their therapeutic management. While ES is managed by neoadjuvant chemotherapy followed by surgery, RMS is treated with upfront surgery, reiterating the importance of an accurate diagnosis. On IHC, ARMS is immunopositive for Desmin, Myogenin, and MyoD1, while characteristically negative for NKX2.2. However, a few other MSRCTs can also show rhabdomyoblastic differentiation, as evidenced by positive myogenic markers, such as mesenchymal chondrosarcoma, which is also known to express NKX2.2.[3]

This makes additional IHC workup imperative when encountering an equivocal immunoprofile, as seen in this case. Recently, MUC4 has been described as a useful adjunct marker for the diagnosis of Alveolar RMS (fusion-positive),[4] which was also used in this case. Although not targetable, cytoplasmic overexpression of ALK protein is known to occur in the vast majority of ARMSs, making it another useful marker in the diagnostic armamentarium.[5]

The presented case highlights the significance of applying comprehensive IHC panel when dealing with MSRCTs of bone and soft tissue. MUC4 is ostensibly a very useful IHC marker and can be explored in further studies as a surrogate for fusion-positive alveolar RMS and to distinguish it from the fusion-negative embryonal RMS, due to distinct prognostic and therapeutic implications—especially in resource-limited centers where specialized molecular techniques, such as BA-FISH or next generation sequencing, are not available.

Authors' Contributions

S.P. and P.C. performed the histological examination. S.J. was involved with the treatment and follow-up of the patient. R.O. was responsible for the radiological workup. S. P. and P.C. wrote the main manuscript text. All authors reviewed and approved the final manuscript.

Patient Consent

Patient consent has been taken.

Conflict of Interest

None declared.

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