

Diagnostic Utility of NKX3.1 in Mesenchymal Chondrosarcoma on Needle Biopsy: A Case Report of a Supraclavicular Soft Tissue Mass with Literature Review

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ABSTRACT

We report a case of young female in her 20s who presented with a supraclavicular soft tissue mass. Diagnostic biopsy showed a malignant round cell tumor with areas of spindling and hyalinized stroma. The utilization of an immunohistochemistry panel revealed positive results for NKX2.2 and CD99 expression. This positivity led to the consideration of a differential diagnosis of Ewing sarcoma, *EWSR1::NFATC2*-rearranged sarcoma, and mesenchymal chondrosarcoma for further assessment. On further immunohistochemistry with NKX3.1 and *EWSR1* break-apart fluorescent in situ hybridization analysis, a diagnosis of mesenchymal chondrosarcoma was rendered which was later on confirmed with biphasic histology on excision specimen. NKX3.1 is a useful immunohistochemistry marker to resolve the differentials when dealing with undifferentiated small round cell sarcoma of bone and soft tissue, especially on a needle biopsy.

Keywords: Ewing sarcoma, *EWSR1::NFATC2*-rearranged sarcoma, Mesenchymal Chondrosarcoma, NKX3.1, Soft tissue

INTRODUCTION

Mesenchymal chondrosarcoma (MC), first described by Lichtenstein and Bernstein, is a high grade malignant mesenchymal tumor involving bones and soft tissues (1). MC is a rare tumor accounting for 2-4% of all chondrosarcomas with peak incidence in 2nd-3rd decade of life without any significant sex predilection. About two-third of the cases arise in bones while the remaining cases are seen in somatic soft tissue of which meninges are the most common site (2-5).

MC have characteristic biphasic histomorphology comprising of variable mixture of well differentiated hyaline cartilage admixed with small to intermediate sized poorly differentiated round cells and hemangiopericytomatous vasculature (2-5). In diagnostic needle biopsy, the round cell tumor component is often sampled and often construed as any other malignant round cell tumor (MRCT). Ewing sarcoma (ES) and *EWSR1::NFATC2*-rearranged sarcoma are the most pertinent differentials of MC. With immunohistochemistry (IHC), all these three entities will show CD99 and NKX2.2 immunopositivity. Recently, NKX3.1 has been described as a useful diagnostic IHC marker for diagnosing MC and *EWSR1::NFATC2*-rearranged sarcoma (3,4). MC is characteristically defined by a recurrent

HEY1::NCOA2 rearrangement representing an in-frame fusion at the mRNA level (2,3,5).

We present a rare case of MC of supraclavicular soft tissue region where NKX3.1 proved to be a potentially useful marker for establishing the diagnosis on needle biopsy.

CASE REPORT

A 28-year-old female with no significant past or family history presented with gradually increasing swelling in the left supraclavicular region for 6 months with the recent onset of pain. On physical examination, no lymphadenopathy was noted. Magnetic resonance imaging upper chest revealed a well-defined lobulated solid mass, 3.9 cm in maximum dimension in the subcutaneous plane adjacent to the clavicle, with no evidence of bone erosion (Figure 1).

Diagnostic needle biopsy was done and histopathology examination (HPE) revealed a MRCT with focal areas showing oval to spindle cells set in a hyalinized stroma and few mitoses (Figure 2A-D). The primary panel of IHC markers revealed expression of CD99 (Figure 3A), NKX2.2 (Figure 3B) while the tumor was negative for desmin, myoD1, LCA, pancytokeratin & SSX18 which ruled out rhabdomyosarcoma, non-Hodgkin lymphomas, and poorly differentiated

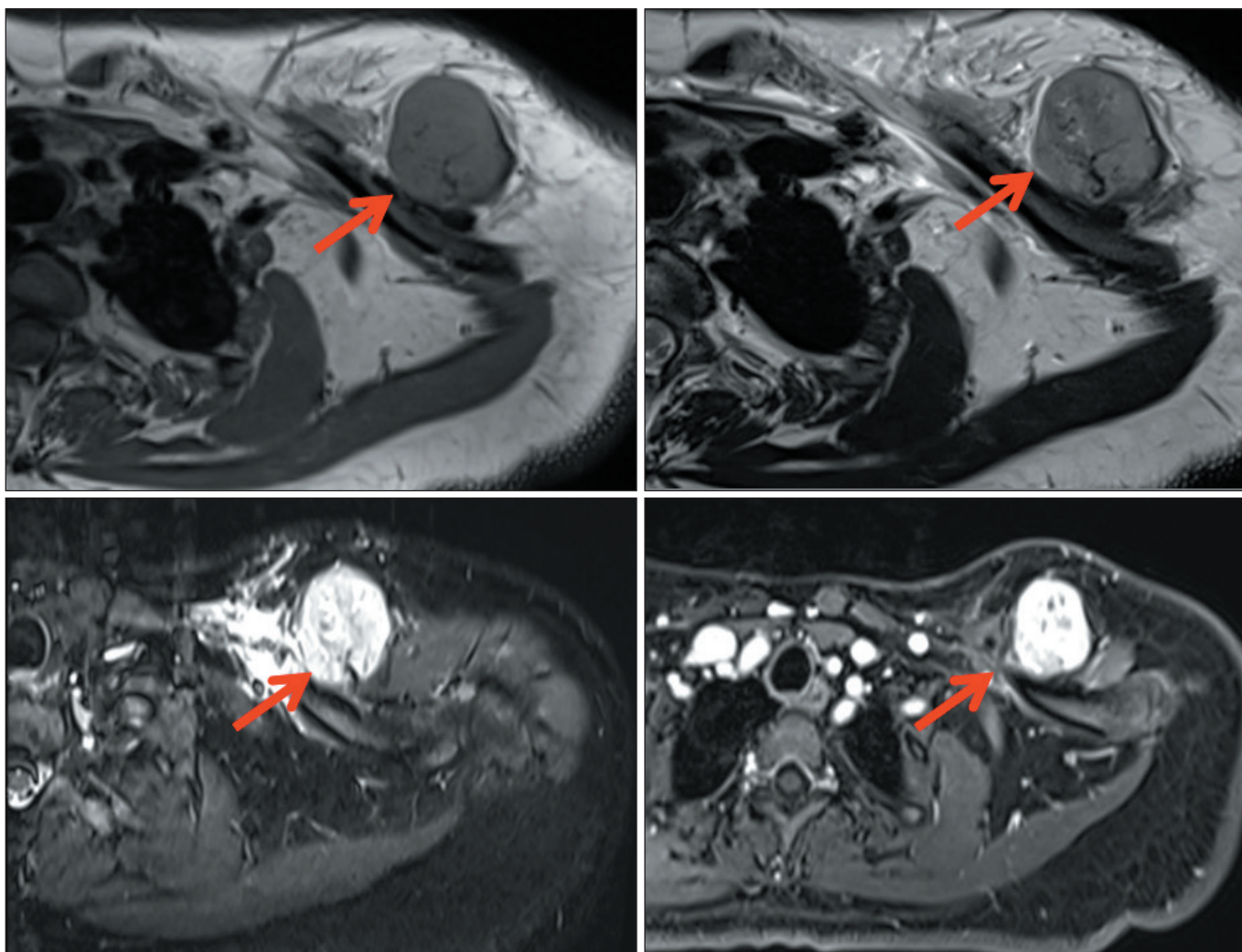


Figure 1: Axial T1, T2, STIR and T1+c images showing a well-defined lobulated solid mass (~3.3x2.5x3.9cm) anterior to the left clavicle, predominantly in the subcutaneous plane (arrows). It is T1 intermediate and T2/STIR hyperintense with marked enhancement on post contrast study. No evidence of bone erosion/periosteal thickening seen.

synovial sarcoma. In view of combined CD99, NKX2.2 positivity, the differentials considered were ES, MC, and *EWSR1::NFATC2*-rearranged sarcoma. NKX3.1 (Figure 3C) and SOX9 was done and showed diffuse nuclear positivity and ruled out classic ES. Subsequently, *EWSR1* gene rearrangement was negative on break-apart fluorescent in situ hybridization (break-apart FISH) (Figure 3D), which ruled out *EWSR1::NFATC2*-rearranged sarcoma and a final diagnosis of MC was suggested. The case was discussed at a multidisciplinary tumor board meeting and excision of the mass was done thereafter.

Grossly, the tumor was relatively well circumscribed with lobulated outlines and the cut surface was homogeneous grey-white with a glistening appearance (Figure 4A). On HPE, the tumor was lobulated and consisted of MRCT in sheets with juxtaposed discrete area of mature hyaline

cartilage (Figure 4B). A hemangiopericytoma-like vascular pattern was well evident and endochondral ossification was seen in occasional foci (Figure 4C-D). Hence, the final diagnosis of MC was established.

The patient was advised only adjuvant radiation therapy (60 Gy in 30 fractions) to the post-operative bed which she underwent at her native place. The patient is now on 10 months of follow-up with no evidence of disease.

DISCUSSION

MC is a rare and aggressive MRCT of bones and soft tissue, the diagnosis of which can be at times very challenging given its rare occurrence and especially when needle biopsy sample extracts only MRCT. Thus, the differential diagnosis covers the spectrum of MRCT in a relevant clinical and radiological context.

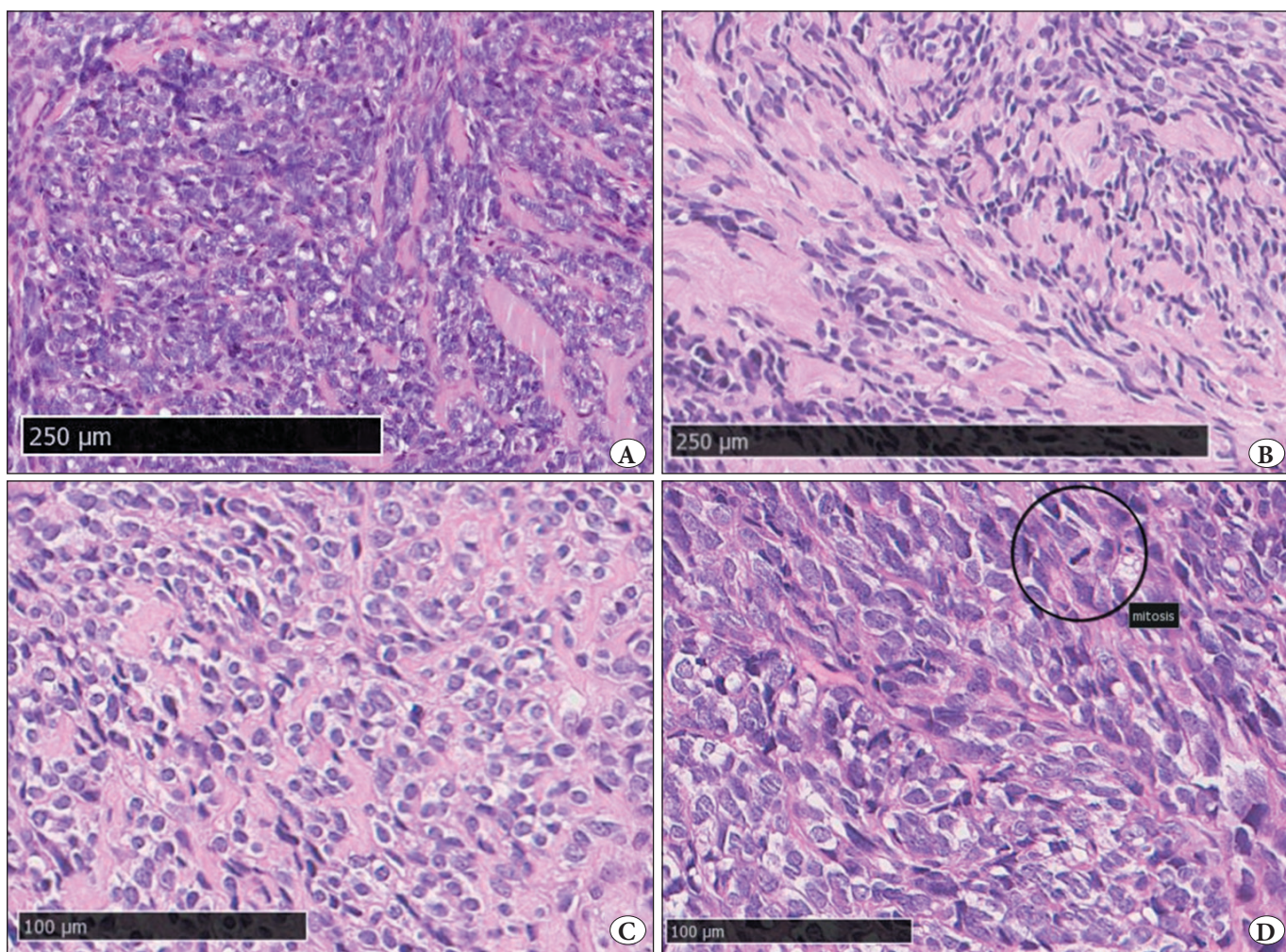


Figure 2: Histology of mesenchymal chondrosarcoma on small biopsy. **A)** Morphology of a malignant round cell tumor. **B)** Areas of spindled tumor cells within hyalinized stroma were noted. **C)** Focal areas showed stroma mimicking osteoid-like material. **D)** Few mitotic figures (circle) were seen in the tumor.

In the present case, the relevant differentials on needle biopsy were ES, rhabdomyosarcoma, poorly differentiated synovial sarcoma, and MC. The combined CD99 and NKX2.2 positivity prompted a diagnosis of ES. However, the presence of foci exhibiting oval to spindled blue tumor cells in hyalinised stroma provoked us to rule out MC as well. Although combined positivity of NKX2.2 and CD99 is highly sensitive and specific for the diagnosis of ES in a suitable clinical context (6,7), MC also exhibits the same immunoprofile (2,6,7).

NKX3.1 has recently emerged as a new, potentially useful marker for the diagnosis of MC (3). Syed et al. (3) evaluated NKX3.1 immunopositivity in 21 cases of MC and 32 other cases of MRCT. They found NKX3.1 positive in 66.7% of the cases (14 out of 21) of MC while all other MRCT cases were negative. Glauser et al. (5) have found NKX3.1

positive expression in 35.9% of the cases (14 out of 39) of MC while all 210 cases included in the sarcoma tissue microarray were negative. Wang et al. (8) have found 100% sensitivity for NKX3.1 (positive in all 12 cases) in the diagnosis of MC. Besides MC, the utility of NKX3.1 immunopositivity has also been described in *EWSR1::NFATC2*-rearranged sarcoma by Yoshida et al. (4). The authors found NKX3.1 immunopositivity in 82% (9 out of 11) cases of *EWSR1::NFATC2*-rearranged sarcoma (diffuse and moderate to strong intensity) while 100% (all 12 cases) of MC cases were immunopositive (4). All the remaining 156 mesenchymal tumors (including 20 cases of ES) were negative for NKX3.1, except for one case of osteosarcoma which showed focal (10%) immunopositivity (4).

SOX9, like NKX3.1, was initially proposed as a reliable marker of chondrogenic differentiation in MC; however it

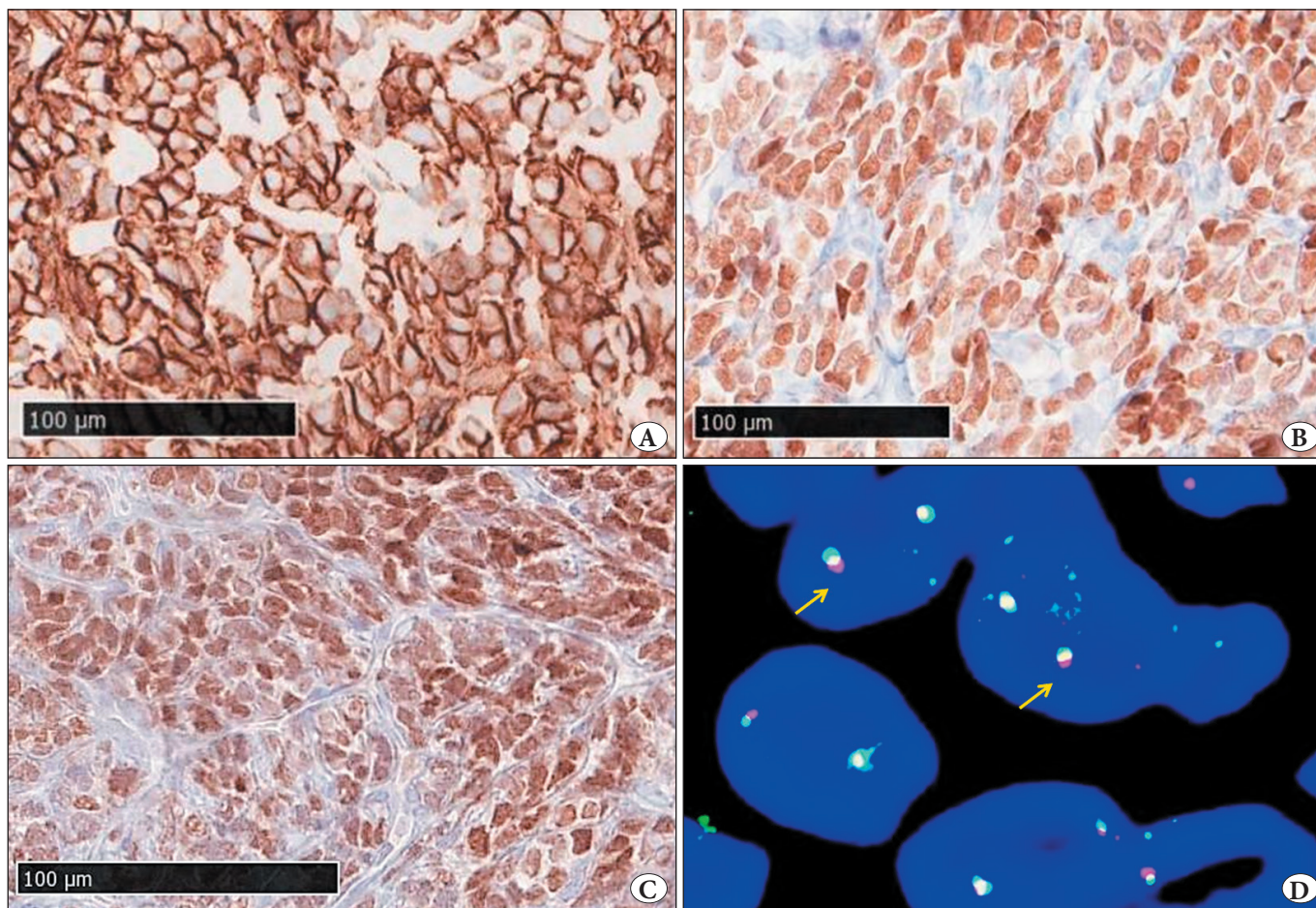


Figure 3: Immunohistochemistry of mesenchymal chondrosarcoma. A) Tumor cells showed strong membranous expression of CD99. B) Tumor cells showed diffuse nuclear expression of NKX2.2. C) Tumor cells showed diffuse nuclear expression of NKX3.1. D) Break-apart fluorescent in situ hybridization analysis was negative for *EWSR1* gene rearrangement (arrows).

has been shown to lack specificity, as its immunorexpression can be observed in various non-chondrogenic soft tissue tumors (9).

The *HEY1::NCOA2* rearrangement is a diagnostic hallmark for MC; however, this test has limited accessibility in laboratories and should therefore be considered only in selected cases. In the present case, a combined positivity of CD99, NKX2.2 with concomitant diffuse NKX3.1 immunorexpression largely ruled out ES. However, *EWSR1::NFATC2*-rearranged sarcoma and MC were still differentials. Clinically, it is prudent for oncologist and pathologist to differentiate between these two entities by *EWSR1* gene rearrangement by break-apart FISH. *EWSR1::NFATC2*-rearranged sarcoma will receive preoperative or adjuvant chemotherapy (VAC-IE regimen) while there is no cogent evidence of the benefit of adjuvant chemotherapy in MC. However, adjuvant radiation therapy is advocated. Therefore, we performed *EWSR1* gene rearrangement by break-apart FISH,

which was negative, and hence we suggested the final diagnosis of MC in needle biopsy.

On resection, the classical biphasic features were demonstrated on HPE (Figure 4B). Hence, in the present case NKX3.1 was of importance in navigation for the final diagnosis of MC in the needle biopsy consisting of only a round cell tumor component. The diagnosis of MC in diagnostic biopsy is also complicated by its enigmatic IHC profile as it may frequently show aberrant immunorexpression for desmin, myoD1, and myogenin, which may prompt an erroneous diagnosis of rhabdomyosarcoma (10,11). This further underscores the urgent need for a specific IHC marker for MC, since it is the only entity in the differentials of MRCT in which chemotherapy is not advocated as per the current therapeutic guidelines, and hence a misdiagnosis can result in unwarranted toxic effects of chemotherapy.

To conclude, NKX3.1 is a useful IHC marker to resolve the differentials when dealing with undifferentiated small

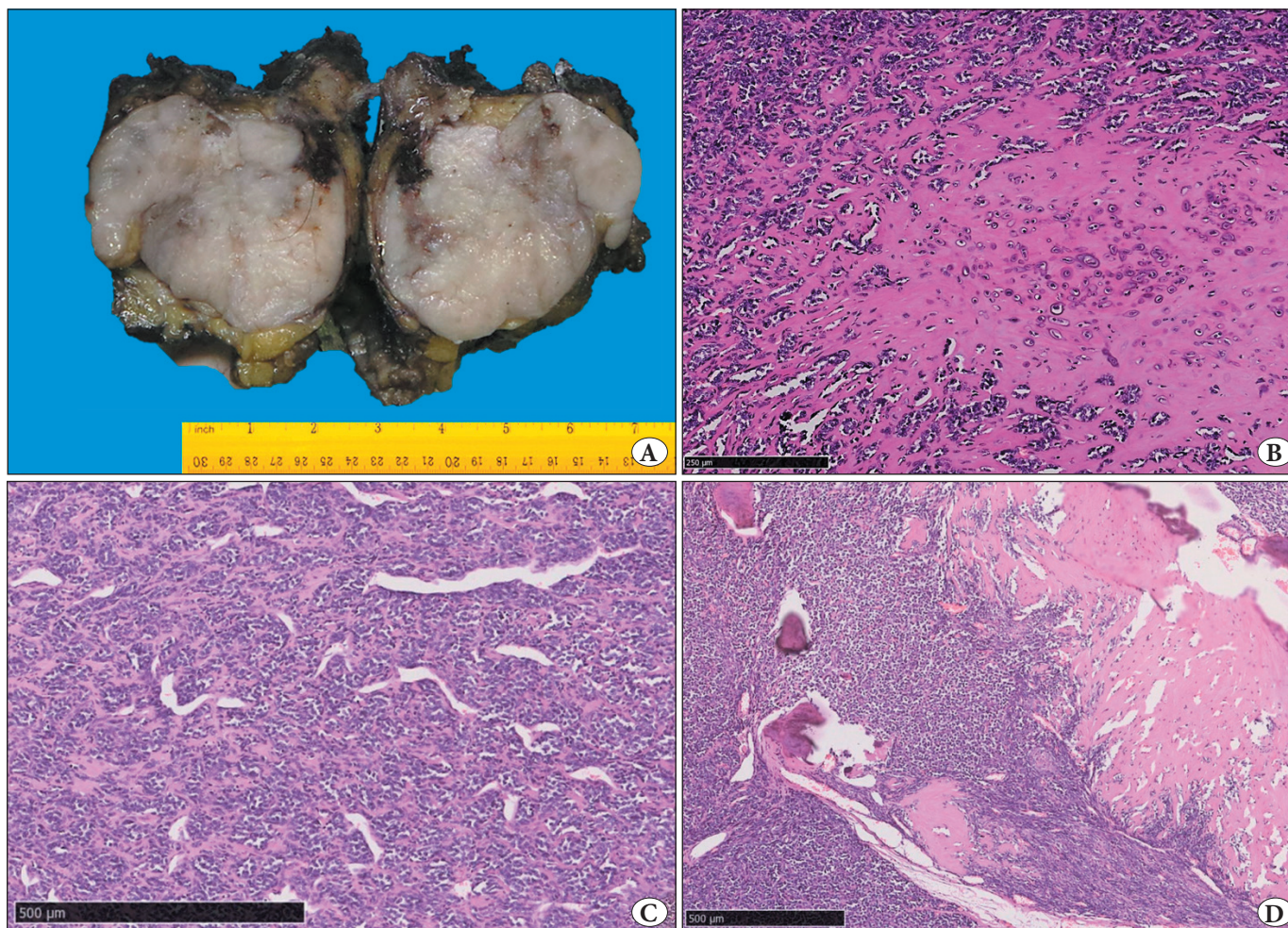


Figure 4: Wide local excision of mesenchymal chondrosarcoma A)Gross image showed a well circumscribed, lobulated, grey-white tumor. B) Characteristic biphasic histology of mesenchymal chondrosarcoma. C) Hemangiopericytomatous vasculature in the tumor. D) Areas of endochondral ossification in hyaline cartilage nodules.

round cell sarcoma of bone and soft tissue, especially in a needle biopsy that may not sample the cartilaginous component, and it has the potential to obviate the need for identifying a fusion transcript. However, a key limitation is that NKX3.1 can be expressed in *EWSR1::NFATC2*-rearranged sarcoma, necessitating additional molecular work-up with *EWSR1* break-apart FISH, as distinguishing these two entities is critical due to their distinct therapeutic and prognostic implications.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Ethics Approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Institutional Review Board (Rajiv Gandhi Cancer Institute & Research Centre); vide the ethical approval letter number RES/SCM/60/2023/74

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Consent for Publication

Consent for publication was obtained for every individual person's data included in the study.

Availability of Data and Material

Availability of data and material is possible upon reasonable request, deidentified for maintenance of anonymity and compliance with IRB approval.

Authorship Contributions

Concept: **SP, DB**, Design: **SP, DB**, Data collection and/or processing: **SP, DB, HR, AS, RO, VR, UB**, Analysis and/or interpretation: **SP, DB, HR, AS, RO, UB**, Literature search: **SP, DB**, Writing: **SP, DB**, Approval: **SP, DB, HR, AS, RO, VR, UB, AM**.

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