Content available at: https://www.ipinnovative.com/open-access-journals

IP Archives of Cytology and Histopathology Research

Journal homepage: https://www.achr.co.in/

Case Report

Is CD99 a culprit in misdiagnosis? A tell-tale of two cases

Anju Shukla[1]*, Zoya Qamar[1]

Dept. of Laboratory Medicine, Max Super speciality Hospital, Lucknow, Uttar Pradesh, India

Abstract

CD99 is a transmembrane protein, a product of the MIC2 gene with various functions and limited diagnostic utility, routinely used in laboratories for the differential diagnosis of small blue cell tumors. It is used in establishing the diagnosis of Ewing's sarcoma/PNET where it consistently shows strong membranous staining on immunohistochemistry. However, CD99 has high negative and low positive predictive values. Therefore, if CD99 is negative, the likelihood of Ewing's sarcoma/PNET is low. Conversely, if it is positive, it is not specific to the Ewing Sarcoma family of tumors, as this antigen's expression is seen in a wide variety of tumors. Thus for confirmation cytogenetic examination for reciprocal translocation t(11;22)(q24;q12) or t(21;22)(q22;q12) which results in the fusion of EWSR1 gene with FLI1 or ERG genes are essential. Here, I describe two cases where the diagnosis of Ewing's sarcoma was made based on immunohistochemistry. In both cases, the EWSR1-FLI1 fusion was found to be negative. Further molecular testing revealed synovial sarcoma in one case, while total resection of the tumor revealed the morphology of mesenchymal chondrosarcoma in the other case. These cases highlight that strong membranous immunostaining of CD99 with NKX2.2 positivity, which is highly specific for Ewing's sarcoma, is insufficient for diagnosis. Round cell sarcoma cases should be confirmed with molecular testing before starting chemotherapy.

Keywords: CD99, Ewings sarcoma, NKX2.2

Received: 10-01-2025; **Accepted:** 30-01-2025; **Available Online**: 22-04-2025

1. Introduction

Immunohistochemistry (IHC) is a widely used ancillary technique used by surgical Pathologists for cell and tumor typing and guiding in therapy and prognosis.[1] There is an increasing demand for IHC in the present era of personalized medicine. IHC has always been defined by sensitivity and specificity. In IHC It is the amount of protein of interest detected and the degree to which the antibody binds to the protein is determined while molecular tests are supposed to be more accurate because they detect specific DNA or RNA sequences.[2]

Diagnosis of a tumor should always be based on morphology on tissue sections stained by Hematoxylin and Eosin stain, supported by special stains with confirmation by immunohistochemistry and/or molecular testing.

Small round cell tumor is a common diagnosis on morphology and currently, a battery of immunohistochemistry supported by molecular testing is used for the diagnosis. It is dangerous to use immunohistochemistry alone to conclude.

Here we are describing two cases with morphological diagnosis of malignant small round cell tumor that were misdiagnosed as Ewing sarcoma based on IHC and the master of the orchestra in both the cases was CD99, which along with many other markers failed to diagnose tumors correctly.

2. Case 1

A 45-year-old men presented with severe backache and pain around the right hip joint seven years ago. An MRI of the spine confirmed the collapse of the second lumbar vertebra with bony infiltration. Serum protein electrophoresis (SPE) with immunofixation electrophoresis (IFE) showed an M band corresponding to IgG and kappa. M spike corresponding to 2.39 mg/dl and serum immunoglobulin revealed increased immunoglobulin IgA 1650 mg/dl (normal range: 70-374

*Corresponding author: Anju Shukla
Email: anjushukla2012@gmail.com

http://doi.org/10.18231/j.achr.2025.005

mg/dl) with a corresponding decrease in levels of IgG and IgM. Bone marrow aspiration and biopsy revealed more than 20% plasma cells and Beta-2 microglobulin levels greater than 4 mg/L (normal range: 1.1 – 2.4 mg/L). Free kappa light chain was 331 mg/L (normal range: 3.3-19.4 mg/L) and the kappa/lambda ratio was increased to 54.6 (normal range: 0.26-1.65) He was diagnosed with Ig kappa multiple myeloma and treated with Bortezomib, Zoledronic acid, Dexamethasone, and Thalidomide.

Eight cycles of Bortezomib were administered. A repeat SPE with immunofixation showed no "M" band, and bone marrow examination revealed no evidence of residual disease. The patient was started on the CHOP regimen, and after two cycles, he was evaluated for an autologous stem cell transplant (ASCT). High-dose melphalan followed by an autologous stem cell transplant was performed, and the patient was discharged in stable condition. He was on regular follow-ups with blood counts, SPE with IFE, immunoglobulin levels, free light chain assay, and Beta-2 microglobulin.

During follow-up, six years after the ASCT, a palpable abdominal mass was found on the right side. Bone marrow examination showed three percent plasma cells, and immunofixation showed no "M" band. Contrast Axial images showing an enlarged right kidney with heterogenous enhancement with nodular soft tissue thickening extending in the perinephric region with interface loss [**Figure 1**a]. Angiography shows areas of abnormal vascularity in the right kidney with pooling of contrast suggestive of neovascularization [**Figure 1**b]. The mass was seen to adhere to surrounding structures, including the liver, duodenum, and colon.

A core biopsy of the renal mass confirmed a high-grade small round cell tumor [**Figure 1**c and d]. On immunohistochemistry, CD99 was strongly positive with a membranous pattern in tumor cells [**Figure 2**a] while tumor cells were negative for Tdt, CD45 [**Figure 2**b], CD20, CD3, AE1/AE3 [**Figure 2**c], and Chromogranin. Vimentin and NKX2.2 were also positive, and BCL-2 and synaptophysin were focally positive. A provisional diagnosis of Ewing's sarcoma was made.

The patient went to another oncology center where immunohistochemistry was repeated, and the diagnosis of Ewing sarcoma was again confirmed based on IHC, leading to the initiation of chemotherapy. When there was no response to treatment, EWS-FLI-1 translocation testing by FISH was performed, and the result was negative. This prompted consideration of another possibility, and further testing for the chromosomal translocation t(X;18)(p11;q11) was conducted, which came back positive, confirming the diagnosis of synovial sarcoma. Unfortunately, the patient succumbed to cardiorespiratory arrest.

3. Case 2

A 31-year-old women presented with pain and swelling in the right hip and thigh. A Tru-cut biopsy was performed on both the right iliac bone and the right thigh. Histology from both biopsies revealed solid sheets of undifferentiated cells with a hemangiopericytoma-like vascular pattern, leading to a diagnosis of round cell sarcoma with a hemangiopericytoma-like vascular pattern, suggestive of mesenchymal chondrosarcoma. Immunohistochemistry, performed at an outside centre, showed positivity for CD99 (strong, membranous) and NKX2.2, while S-100, SATB2, Pan CK, Desmin, and CD45 were negative. A diagnosis of Ewing's sarcoma was given, and molecular testing for the EWSR1 gene was advised.

In the meantime, chemotherapy with a specific protocol of alternating vincristine/doxorubicin/cyclophosphamide and ifosfamide/etoposide (VDC/IE) was started. The EWSR1 gene was found negative on molecular testing, but chemotherapy continued, resulting in only a 30% reduction in the tumor after 9 cycles of therapy. Consequently, tumor reduction surgery was performed, involving resection of the right thigh sarcoma, removal of the right iliac bone sarcoma, and lymph node dissection. The large tumor mass was sent for histopathological evaluation.

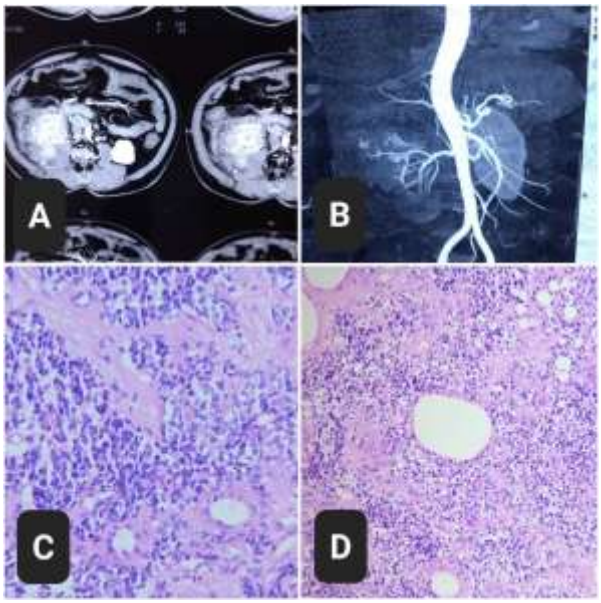

Figure 1: A: Contrast Axial images showing enlarged right kidney with heterogenous enhancement with nodular soft tissue thickening extending in perinephric region with loss of interface; **B:** Angiography shows areas of abnormal vascularity in right kidney with pooling of contrast suggestive of neovascularization; **C:** Cellular tumor in sheets separated by vascular channels, hematoxylin and eosin x20; **D:** Infiltrating the surrounding adipose tissue hematoxylin and eosin x10.

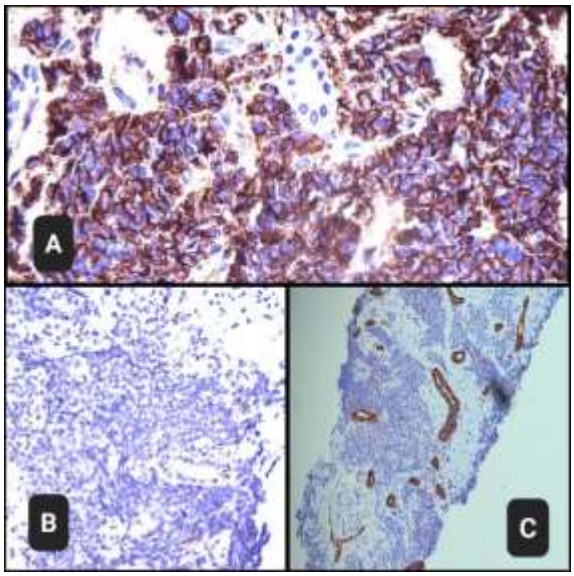

Figure 2: A: Strong membranous CD99 immunostaing x20; **B:** No staining with CD45 x10; **C:** No Pancytokeratin staining in tumor cells, internal control (tubules) showing staining x4.

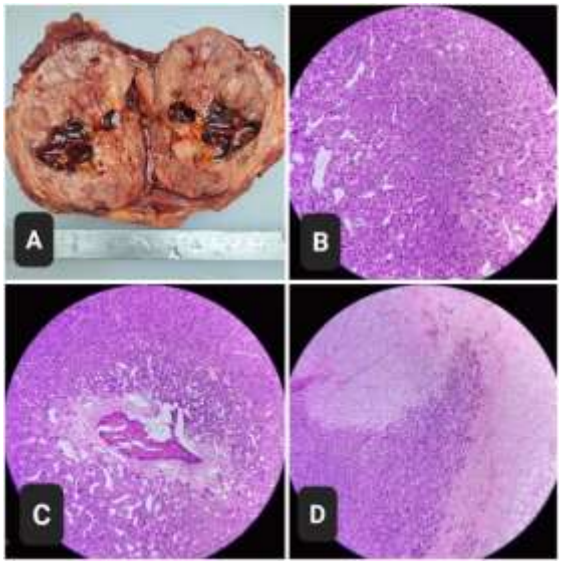

Figure 3: A: Fleshy cut surface with hemorrhagic cystic areas; **B:** Cellular tumor with hemangiopericytoma like growth pattern hematoxylin and eosin x4; **C:** Tumor infiltrating bony trabeculae hematoxylin and eosin x4. (D) Biphasic tumor with cellular undifferentiated and chondroid areas hematoxylin and eosin x4.

Gross examination of the thigh mass revealed a globular, grey-brown tissue piece measuring 15.0 x 11.0 x 10.0 cm, with a central fleshy mass, gritty to cut, measuring 11.0 x 11.0 x 10.0 cm [**Figure 3**a]. The mass exhibited multiple cystic foci filled with blood clots and areas of necrosis. A separate iliac bone specimen with soft tissue, measuring 10.5 x 6.5 x 4.5 cm, was also involved by the tumor. Sections from both the thigh mass and the iliac bone with soft tissue extension revealed a biphasic pattern: small, undifferentiated round to spindled cells, and interspersed blood vessels [**Figure 3**b] admixed with abundant blue-grey cartilage matrix production, and irregularly shaped lobules of cartilage [**Figure 3**d] Marked cellular atypia, bizarre giant forms, multinucleated forms, and numerous typical and atypical mitoses were observed. Enchondral ossification and permeation of the tumor between pre-existing bony trabeculae were seen [**Figure 3**c]. All the lymph nodes revealed reactive hyperplasia only. Histomorphology was characteristic of mesenchymal chondrosarcoma. Unfortunately, the patient died on the second postoperative day due to cardiac arrest.

4. Discussion

Small round cell tumors (SRCT), also known as small round blue cell tumors (SRBCT), have a similar histologic appearance: small, round, relatively undifferentiated cells. This diagnosis is challenging and includes Lymphoma, Ewing's sarcoma, rhabdomyosarcoma, synovial sarcoma, retinoblastoma, neuroblastoma, hepatoblastoma, Wilms' tumor, small cell osteogenic sarcoma, undifferentiated hepatoblastoma, granulocytic sarcoma, and intra-abdominal desmoplastic small round cell tumor.[3] Out of this long list non-Hodgkin lymphoma is the most common small blue cell tumor.[4] Immunohistochemistry is primarily used for characterization in SRBCT and CD99 is used as a baseline marker along with CD45, CD20, CD3, keratin, EMA, desmin, synaptophysin, chromogranin, myogenin, CD56, etc.

CD 99 is a cell surface glycoprotein encoded by MIC 2 gene located on short arm of X and Y chromosome and is consistently expressed in by the cells of Ewing Sarcoma/ Primitive neuroectodermal tumor.[5] Only strong, diffuse and membranous staining is considered positive for Ewing sarcoma and acute lymphoblastic leukemia / lymphoma.[6]

However CD99 expression is not specific for Ewing Sarcoma as many diagnostic entities showed CD99 positivity and the list is ever- expanding, thus now CD99 has been referred to as staining "99 different things".

Among small round cell tumors, CD99 positivity seen in synovial sarcoma, mesenchymal chondrosarcoma, and lymphoblastic lymphoma, complicating its use as a sole diagnostic marker.[7]

Besides CD99, antibodies to FLI1 (Friend leukemia integration 1) and ERG may be positive in Ewing sarcoma due to EWSR1-FLI1 and, less commonly, EWSR1-ERG fusions.[8] Given the increasing number of tumors found to express FLI1, the use of more specific markers and molecular confirmation is necessary for the accurate diagnosis of Ewing sarcoma.[9] Similarly, ERG immunostaining is positive in only 5% of Ewing sarcoma cases, and its primary diagnostic utility lies in vascular tumors, where it is currently considered the best available marker for endothelial differentiation.[10]

NKX2.2 is another valuable marker for Ewing sarcoma, with a sensitivity of 93% and a specificity of 89%.[11]

However, it is not entirely specific for Ewing sarcoma, as it can also be positive in a subset of other tumors, including neuroendocrine tumors and epithelial tumors with neuroendocrine differentiation, such as small cell carcinoma, well-differentiated NET, and Merkel cell carcinoma. Additionally, it is seen in some mesenchymal tumors, including subsets of synovial sarcomas, mesenchymal chondrosarcomas, and spindle cell/sclerosing rhabdomyosarcoma.[12] NKX2.2 positivity has also been observed in malignant melanoma and olfactory neuroblastoma.[11] Therefore, NKX2.2 should be used in conjunction with other immunohistochemical markers and confirmatory molecular tests to ensure diagnostic accuracy.

In summary, the combination of CD99, NKX2.2, and vimentin is not solely diagnostic for Ewing sarcoma, as demonstrated in both of our cases. Shibuya et al. showed that the combination of NKX2.2 and CD99 improved specificity to 98%, with rare expression of both markers in non-Ewing sarcoma tumors. In their study, these exceptions included two cases: one of mesenchymal chondrosarcoma and another of small cell carcinoma.[13] They concluded and emphasized that NKX2.2 is a valuable immunohistochemical marker for Ewing sarcoma and that the combination of CD99 and NKX2.2 serves as a powerful diagnostic tool to distinguish Ewing sarcoma from other small round cell tumors (SRCTs).

When we critically analyzed our first case, where the standard treatment protocol for Multiple Myeloma (MM) was followed, the patient, who was in good performance status, underwent high-dose chemotherapy followed by autologous stem cell transplant (ASCT). The patient developed a second malignancy (SM) six years later. The incidence of SM in MM patients has been estimated through population-based registry studies, retrospective analyses, and prospective clinical trials, consistently showing a low occurrence rate of 5–7%, with standardized incidence rates (SIR) between 0.98 and 1.26.[14] These second malignancies primarily consist of hematological cancers, with three main types occurring after transplant: therapy-related blood cancers such as acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), lymphomas, primarily post-transplant lymph proliferative disorder (PTLD), and, more rarely, solid tumors. Reported solid tumors include melanoma and other skin cancers, as well as cancers of the oral cavity, head and neck, brain, liver, uterine cervix, thyroid, breast, lung, and potentially the gastrointestinal tract.[15] The risk decreases after five years.

Our patient presented with a right renal mass six years post-ASCT and was initially suspected to have a lympho proliferative disorder, so the primary diagnostic panel was targeted accordingly. The exclusion of hematolymphoid malignancy and positive markers for CD99 and NKX2.2 supported the diagnosis of Ewing sarcoma; however, the positivity of Bcl-2, which is found in 79–100% of synovial sarcoma cases, was overlooked in this case.[16] A literature search did not reveal any cases of synovial sarcoma occurring after ASCT.[17]

The use of limited markers and the paucity of literature on the occurrence of rare tumors as second primary malignancies contributed to the misdiagnosis. The positivity for vimentin, CD99, NKX2.2, and synaptophysin pointed towards Ewing sarcoma; however, the significance of Bcl-2 positivity was not interpreted and TLE1, a relatively sensitive and specific marker for synovial sarcoma, was not performed.[18]

In the second case, the major determinant for misdiagnosis was the initial small tru-cut biopsy, which only showed a cellular, undifferentiated neoplasm. Immunohistochemistry (IHC) suggested Ewing sarcoma after excluding other small round cell tumors. The diagnosis was based on CD99 and NKX2.2 positivity, but both markers are also positive in mesenchymal chondrosarcoma.[19]

These two cases highlight the necessity of confirming a diagnosis of Ewing sarcoma through molecular methods before starting treatment. However, one drawback is that a single translocation is not 100% specific. The most common translocation, t(11;22)(q24;q12), resulting in the EWSR1-FLI1 fusion, is seen in approximately 85-90% of cases. The second most common translocation, t(21;22)(q22;q12), resulting in the EWSR1-ERG fusion, is seen in approximately 5-10% of cases. Rare fusions detected in less than 1% of cases involve ETV1 (7p22), ETV4 (17q21), and FEV (2q35-36). Additionally, two rare translocations involving ERG or FEV with FUS include t(16;21)(p11;q22) FUS-ERG and t(2;16)(q35;p11) FUS-FEV.[20] Thus, all these alterations must be tested before ruling out the possibility of Ewing sarcoma.

5. Conclusion

Immunohistochemistry is a surrogate test for the diagnosis of Ewing's sarcoma; however, confirmation by molecular methods is essential before starting treatment. Due to the poor specificity of CD99, one should avoid making a diagnosis based solely on it and even specific markers, such as NKX2.2 may be non -reliable at times. The availability of numerous IHC markers with overlapping expressions across various tumors, along with the presence of multiple molecular signatures within the same tumor, makes diagnosis complex and poses significant cost-effectiveness challenges, particularly in economically constrained countries like India.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Magaki S, Hojat SA, Wei B, So A, Yong WH. An introduction to the performance of immunohistochemistry. *Methods Mol Biol*. 2019;1897:289–98. doi: 10.1007/978-1-4939-8935-5_25.
2. Gurzu S, Jung I. Immunohistochemistry and molecular biology of cancer. In: Rezaei N, editor. Handbook of cancer and immunology. Cham: Springer; 2022. p. 128-1. doi: 10.1007/978-3-030-80962-1_128-1.
3. Sharma S, Kamala R, Nair D, Ragavendra TR, Mhatre S, Sabharwal R, et al. Round cell tumors: classification and immunohistochemistry. *Indian J Med Paediatr Oncol*. 2017;38(3):349–53. doi: 10.4103/ijmpo.ijmpo_84_16.
4. D'cruze L, Dutta R, Rao S, Anuradha R, Varadarajan S, Kuruvilla S. The role of immunohistochemistry in the analysis of the spectrum of small round cell tumours at a tertiary care centre. *J Clin Diagn Res*. 2013;7(7):1377–82. doi: 10.7860/JCDR/2013/5127.3132.
5. Scotlandi K, Serra M, Manara MC, Benini S, Sarti M, Maurici D, et al. Immunostaining of the p30/32MIC2 antigen and molecular detection of EWS rearrangements for the diagnosis of Ewing's sarcoma and peripheral neuroectodermal tumor. Hum Pathol. 1996;27(4):408-16.
6. Buxton D, Bacchi CE, Gualco G, Weiss LM, Zuppan CW, Rowsell EH, et al. Frequent expression of CD99 in anaplastic large cell lymphoma: a clinicopathologic and immunohistochemical study of 160 cases. *Am J Clin Pathol*. 2009;131(4):574–9. doi: 10.1309/AJCPE68HZXCGWTKK.
7. Pasello M, Manara MC, Scotlandi K. CD99 at the crossroads of physiology and pathology. *J Cell Commun Signal*. 2018;12:55–68. doi: 10.1007/s12079-017-0445-z.
8. Wang WL, Patel NR, Caragea M, Hogendoorn PCW, López-Terrada D, Hornick JL et al. Expression of ERG, an Ets family transcription factor, identifies ERG-rearranged Ewing sarcoma. *Mod Pathol*. 2012;25(10):1378–83.
9. Raza M, Din NU. FLI1. PathologyOutlines.com. Available from: https://www.pathologyoutlines.com/topic/stainsfli1.html. Accessed 2024 Oct 6.
10. Miettinen M, Wang ZF, Paetau A, Tan SH, Dobi A, Srivastava S, et al. ERG transcription factor as an immunohistochemical marker for vascular endothelial tumors and prostatic carcinoma. *Am J Surg Pathol*. 2011;35(3):432-41. doi: 10.1097/PAS.0b013e318206b67b.
11. Hung YP, Fletcher CD, Hornick JL. Evaluation of NKX2-2 expression in round cell sarcomas and other tumors with EWSR1 rearrangement: imperfect specificity for Ewing sarcoma. *Mod Pathol*. 2016;29(4):370–80. doi: 10.1038/modpathol.2016.31.
12. Russell-Goldman E, Hornick JL, Qian X, Jo VY. NKX2.2 immunohistochemistry in the distinction of Ewing sarcoma from cytomorphologic mimics: diagnostic utility and pitfalls. *Cancer Cytopathol*. 2018;126(11):942–51. doi: 10.1002/cncy.22056.
13. Shibuya R, Matsuyama A, Nakamoto M, Shiba E, Kasai T, Hisaoka M et al. The combination of CD99 and NKX2.2, a transcriptional target of EWSR1-FLI1, is highly specific for the diagnosis of Ewing sarcoma. *Virchows Arch*. 2014;465(6):599–605. doi: 10.1007/s00428-014-1627
14. Radivoyevitch T, Dean RM, Shaw BE, Brazauskas R, Tecca HR, Molenaar RJ, et al. Risk of acute myeloid leukemia and myelodysplastic syndrome after autotransplants for lymphomas and plasma cell myeloma. *Leuk Res*. 2018;74:130–6.
15. Danylesko I, Shimoni A. Second malignancies after hematopoietic stem cell transplantation. *Curr Treat Options Oncol*. 2018;19(2):9. doi: 10.1007/s11864-018-0528-y.
16. Krskova L, Kalinova M, Brizova H, Mrhalova M, Sumerauer D, Kodet R. Molecular and immunohistochemical analysis of BCL2, Ki-67, and Cyclin D1 expression in synovial sarcoma. *Cancer Genet Cytogenet*. 2009;193(1):1–8.
17. Costa LJ, Godby KN, Chhabra S, Cornell RF, Hari P, Bhatia S. Second primary malignancy after multiple myeloma: population trends and cause-specific mortality. *Br J Haematol*. 2018;182(4):513–20.
18. Foo WC, Cruise MW, Wick MR, Hornick JL. Immunohistochemical staining for TLE1 distinguishes synovial sarcoma from histologic mimics. *Am J Clin Pathol.* 2011;135(6):839-44. doi: 10.1309/AJCP45SSNAOPXYXU.
19. Dudzisz-Śledź M, Kondracka M, Rudzińska M, Zając AE, Firlej W, Sulejczak D, et al. Mesenchymal chondrosarcoma from diagnosis to clinical trials. *Cancers (Basel)*. 2023;15(18):4581. doi: 10.3390/cancers15184581.
20. Kilpatrick SE, Reith JD, Rubin B. Ewing sarcoma and the history of similar and possibly related small round cell tumors: from whence have we come and where are we going? *Adv Anat Pathol*. 2018;25(5):314-26. doi: 10.1097/PAP.0000000000000203.

Cite this article Shukla A, Qamar Z. Is CD99 a culprit in misdiagnosis? A tell-tale of two cases. *IP Arch Cytol Histopathol Res*. 2025;10(1):18-22.