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Original Article

Role of Nkx2.2 immunohistochemistry in distinguishing Ewing sarcoma from neuroendocrine neoplasms at different sites

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الملخص

أهداف البحث: تُعد ساركوما إيوينغ من الأورام اللحمية عالية الدرجة ذات الخلايا المستديرة، وتُظهر تداخلًا شكليًا نسيجيًا ملحوظًا مع عدد من أورام الخلايا المستديرة الأخرى، وتُعد الأورام العصبية الصمّاوية من أهم التشخيصات التفريقية المشابهة لها. هدفت هذه الدراسة إلى مقارنة التعبير المناعي النسيجي للواسم Nkx2.2 في حالات ساركوما إيوينغ، والأورام العصبية الصمّاوية في مواقع مختلفة، إضافة إلى مجموعة صغيرة من الأورام الميزانشيمية وغير الميزانشيمية، وذلك لتقييم فائدته في التمييز بين هذه الكيانات المرضية.

طرق البحث: أُجريت هذه الدراسة الوصفية الاستعادية خلال فترة 14 شهراً، وشملت 60 حالة، منها 17 حالة من ساركوما إيوينغ، و34 حالة من الأورام العصبية الصمّاوية، و9 حالات من أورام أخرى. جرى استرجاع ومراجعة الشرائح المصبوغة بالهيماتوكسيلين والإيوزين، إضافة إلى الشرائح المصبوغة بالتلوين المناعي النسيجي لـ Nkx2.2، وذلك للحالات التي سبق تشخيصها.

النتائج: أظهر Nkx2.2 حساسية بلغت 88.2% ونوعية بلغت 53.4% في تشخيص ساركوما إيوينغ. كما لوحظ تعبير Nkx2.2 في الأورام العصبية الصمّاوية، وخصوصاً ذات المنشأ الهضمي والبنكرياسي، حيث بلغت الحساسية 52.9% والنوعية 34.6%. وشملت الأورام العصبية الصمّاوية الأخرى الإيجابية لـ Nkx2.2 أورام البروستاتا والجهاز التناسلي الأنثوي والحنجرة. ومن بين الأورام الأخرى، أظهرت الساركوما متعددة الأشكال والميلانوما إيجابية لـ Nkx2.2. في المقابل، كانت غالبية حالات الأورام العصبية الصمّاوية الرئوية، بنسبة 83.3% (6/5)، سلبية لـ Nkx2.2.

الاستنتاج: يُظهر Nkx2.2 حساسية عالية في تشخيص ساركوما إيوينغ وبعض الأورام العصبية الصمّاوية، وخصوصاً ذات المنشأ الهضمي والبنكرياسي. إلا أن انخفاض نوعيته وتفاوت تعبيره بين أنواع مختلفة من الأورام يؤكدان ضرورة استخدامه بحذر وضمن سياق تشخيصي مناسب، وبالاقتران مع واسمات مناعية نسيجية إضافية في الممارسة السريرية.

الكلمات المفتاحية: ساركوما إيوينغ؛ الأورام العصبية الصمّاوية؛ Nkx2.2؛ الساركوما

Abstract

Objectives: Ewing sarcoma is a high grade round cell sarcoma that exhibits considerable histomorphological overlap with other round cell tumors, especially neuroendocrine neoplasms. In the present study, we compared the immunohistochemical expression of Nkx2.2 in cases of Ewing's sarcoma, neuroendocrine neoplasms at different sites, and a small subset of other mesenchymal and nonmesenchymal tumors in order to assess its utility in their distinction.

Materials and methods: This descriptive retrospective study lasted for 14 months and investigated 60 cases, with 17 cases of Ewing sarcoma, 34 cases of neuroendocrine neoplasms and nine cases of other tumors. Hematoxylin and eosin and Nkx2.2 immunohistochemistry-stained slides of previously diagnosed cases were retrieved and reviewed.

Results: Nkx2.2 exhibited sensitivity of 88.2% and specificity of 53.4% in the diagnosis of Ewing's sarcoma. Nkx2.2 expression was also present in neuroendocrine neoplasms (especially those of gastrointestinal and pancreatic origins) with 52.9% sensitivity and 34.6% specificity. Other neuroendocrine neoplasms that were positive for Nkx2.2 included those of prostate, female genital tract, and larynx origins. Among other tumors, pleomorphic sarcoma and melanoma exhibited Nkx2.2

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positivity. Most cases (83.3%, 5/6) of lung neuroendocrine neoplasms were negative for Nkx2.2.

Conclusion: Nkx2.2 exhibited high sensitivity in diagnosing Ewing's sarcoma and certain neuroendocrine neoplasms (especially those of gastrointestinal and pancreatic origins), but its lower specificity and varied expression across other tumor types highlight the need for a cautious and context-specific approach when used in clinical practice.

Keywords: Ewing; Neuroendocrine; Nkx-2.2; Sarcoma

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Introduction

NK2 homeobox 2 (Nkx2.2) is a transcription factor that contains both activation and repressor domains, and it plays critical roles in the differentiation of beta cells within pancreatic islets, enteroendocrine cells in the intestine, and oligodendrocytes in the central nervous system. Nkx2.2 is expressed by endocrine cells located in the villi and deep crypts, and a rare subset of Nkx2.2-positive cells also co-expresses intestinal stem cell markers.[1–3]

In previous studies, Nkx2.2 has been reported as a diagnostically useful immunohistochemical (IHC) marker for Ewing's sarcoma (ES), with sensitivity as high as 93%.[1,4] Nkx2.2 is a downstream target of *EWSR1-FLI1* oncogenic signaling in ES.[4,5] However, the specificity of this marker remains controversial.[5–7]

ES is a high grade round cell sarcoma that exhibits considerable histomorphological overlap with other round cell tumors.[5] Its differential diagnoses include a wide variety of small round blue cell tumors such as other sarcomas, e.g., rhabdomyosarcomas, synovial sarcoma, and desmoplastic small round cell tumor, carcinomas (small cell and Merkel cell), lymphoblastic lymphomas, melanoma, and pediatric tumors (neuroblastoma and Wilms tumor), as well as neuroendocrine neoplasms (NENs), and thus diagnosing ES can pose a considerable challenge and often requires IHC and molecular techniques.[4] Immunohistochemical markers such as CD99 and FLI-1 have been used for the diagnosis of ES, but with suboptimal specificities due to their positive expression in a variety of other tumors.[8–10]

NENs are among the most common histomorphological differentials of ES and the most widely used panel of IHC markers for their diagnosis includes synpatophysin, chromogranin, CD56, and, more recently, INSM1.[11]

In addition to the expression of Nkx2.2 in tumors with neuroendocrine differentiation, studies have shown that ES can express neuroendocrine markers, and thus the IHC overlap in addition to the cytomorphologic overlap can lead to diagnostic difficulties.[4,5,12]

In the present study, we compared the expression patterns of Nkx2.2 IHC in diagnosed cases of ES and NENs at different sites in order to assess the utility of this marker in terms of its sensitivity and specificity at distinguishing the two entities. Most of the available data are derived from western studies and, to the best of our knowledge, the present study is one of the first to be conducted in central India.

Materials and Methods

This descriptive retrospective study was conducted by selecting previously diagnosed cases for a period of 14 months (January 2023 to March 2024) with a total sample size of 60 cases. We included 17 cases of ES (resection = 2, biopsy = 12, paraffin blocks for review = 3), 34 cases of NENs at different sites (resection = 6, biopsy = 21, paraffin blocks for review = 7), and nine cases of mesenchymal and nonmesenchymal tumors (biopsy = 6, paraffin blocks for review = 3). The cases were selected retrospectively from the archives of the department of surgical pathology at our institute. This study included all cases of ES, NENs, and other tumors diagnosed during this period where Nkx2.2 IHC was part of diagnostic panel.

All clinical and radiological details of the cases were retrieved from the hospital records and the Yuvaan + application with the respective server. Two pathologists (SK with MJ) re-evaluated the histopathological slides for all the cases to assess tissue adequacy and confirm the diagnoses, considering the overall clinico-radiological and histomorphological features with supporting IHC markers, including Nkx2.2. Institutional ethics committee (IEC) approval was obtained (IEC NO: 196/2021).

Immunohistochemistry

The clone used for Nkx2.2 IHC was a ready to use Quartett rabbit monoclonal antibody (QR077, manufactured by BIOCYC Ges. F. Biotechnologies & Recyclingverfahren mbH. Am Muhlenberg 4, 14476 Potsdam, Germany) against Nkx2.2, and appropriate positive (pancreas) and negative (skin) controls were also employed. Nuclear Nkx2.2 staining was assessed for each case and scored semi-quantitatively for extent (1+, 1–15%; 2+, 26–75%; or 3+, >75%) and intensity (weak, moderate, or strong) based on microscopic examination by pathologists. Any extent of Nkx2.2 expression was recorded and considered positive. However, the sensitivity, specificity, and positive predictive value were calculated using cases of ES and NENs.

Statistical methods

This study primarily involved categorical variables. Inferential comparisons between groups were performed using the Chi-square test when appropriate. Diagnostic accuracy parameters including sensitivity, specificity, and positive predictive value were calculated by standard contingency table analysis. A p-value <0.05 was considered to indicate a statistically significant difference.

Results

In total, 60 cases were studied, with 17 cases of ES, 34 cases of NENs at different sites, three cases of melanomas of soft tissues, two cases of synovial sarcoma, two cases of T-

cell lymphoblastic lymphoma, and two cases of pleomorphic sarcoma.

Nkx2.2 expression in ES

Among the 17 cases of ES included in this study, positive Nkx2.2 expression was significantly observed in most cases (88.2%; 15/17). Nine (52.9%) cases exhibited strong intensity staining (Figure 1), five (29.4%) cases had moderate intensity staining, and only a single case had weak intensity staining. The remaining two cases were negative for Nkx2.2.

According to the extent of staining, 3+ staining was observed in most cases, i.e., 11/17 cases (64.7%), and 2+ staining was observed in the remaining four cases (23.5%). All cases of ES exhibited positivity for other markers such as CD99 (diffuse, strong, membranous staining) and FLI-1 (diffuse, strong, nuclear staining), supporting the diagnoses. All cases were genetically confirmed and found to have *EWS-FLI1*/t(11; 22) based on molecular studies.

Nkx2.2 expression in NENs

Thirty-four cases of NENs at different sites were included in this study. These comprised 14 cases of gastrointestinal (GIT) (stomach, four; liver, four; gall bladder, two; duodenum, one; sigmoid colon, one; anal canal, one; appendix, one) NENs, four cases of pancreatic NENs, four cases of central nervous system (pituitary, one; clivus, one; spine, two) NENs, six cases of pulmonary(lung) NENs, two cases of female genital tract NENs (ovary, one; uterus, one), one case of prostate NEN, one case of salivary gland NEN, and two cases of larynx NENs.

Eighteen out of 34 (52.9%) cases exhibited Nkx2.2 positivity, where six (17.6%) cases had strong intensity staining, nine (26.4%) cases had moderate intensity staining, and the remaining three (8.8%) cases had weak intensity staining with staining extents of 3+ (10/34; 29.4%) or 2+ (6/34; 17.6%). Sixteen (16/34; 47%) cases were negative for Nkx2.2.

Among the 14 cases of GIT NENs, 12 cases were neuroendocrine carcinomas (NECs) and two cases were neuroendocrine tumors (NETs). Nine cases (NECs = 2, NENs = 7) exhibited nuclear positivity, where four cases had strong intensity staining, three cases had moderate intensity staining, and two cases had weak staining intensity, with staining extents of 3+ (7/9; 77.7%) (Figures 2 and 3), 2+ (one case), or 1+ (one case).

Among four cases of pancreatic NENs, three cases were NECs and one case was NET. Three cases (75%) were nuclear positive for Nkx2.2, whereas one case was negative.

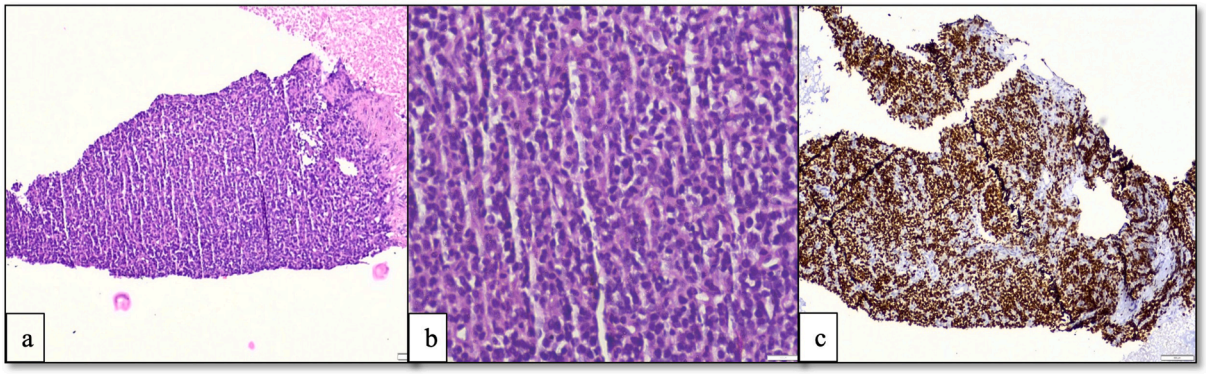


Figure 1: Nkx2.2 in Ewing's sarcoma of femur (biopsy). (a) Ewing's sarcoma: diffuse sheet of tumor (hematoxylin and eosin (H & E), ×100). (b) Uniform round blue cells with high nuclear to cytoplasmic ratio, hyperchromatic nuclei, and scant cytoplasm (H & E, ×400). (c) Nkx2.2 IHC showing diffuse (3+) and strong staining (3,3′-diaminobenzidine, ×200).

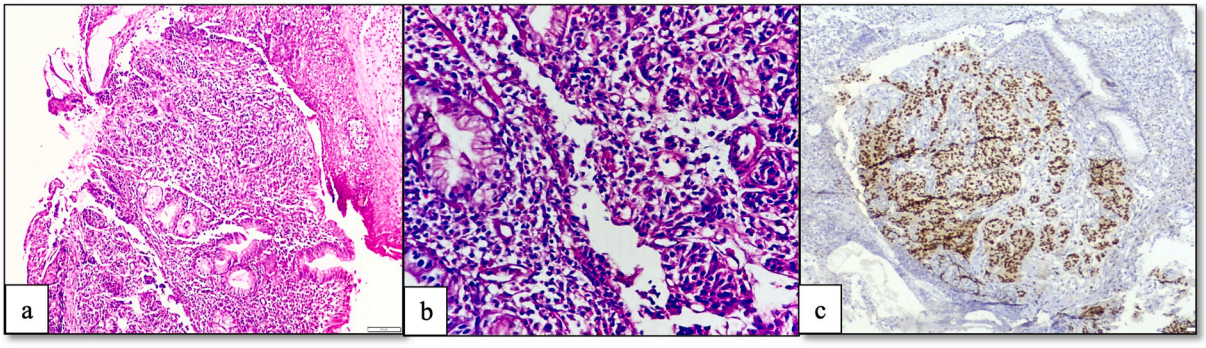


Figure 2: Nkx2.2 in neuroendocrine neoplasm (NEN) of duodenum. (a) NEN of duodenum (hematoxylin and eosin (H & E), ×200). (b) Round cells with hyperchromatic nuclei, forming nests (H & E, ×400). (c) Nkx2.2 IHC exhibiting diffuse (3+) and strong staining (3,3′-diaminobenzidine, ×200).

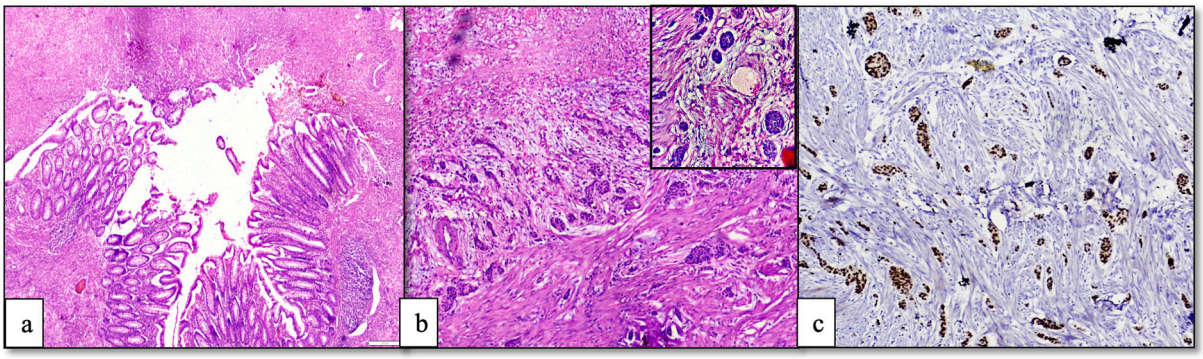


Figure 3: Nkx2.2 in appendiceal neuroendocrine neoplasm. (a) Appendix: luminal epithelium (hematoxylin and eosin (H & E), ×40). (b) Muscularis propria showing NET in appendix forming small nests (H & E, ×200). Inset: round tumor cells with a high nuclear cytoplasmic ratio, vesicular nuclei, salt and pepper chromatin, and scant cytoplasm (H & E, ×400). (c) Nkx2.2 IHC exhibiting diffuse (3+) and strong nuclear staining (3,3′-diaminobenzidine, ×200).

Among the three positive cases (NECs = 2, NEN = 1), the staining intensity was strong for a single case with an extent of 3+, and the staining intensity was moderate for the remaining two cases, with staining extents of 3+ (one case) or 2+ (one case) (see Figure 4).

Among the four cases of central nervous system NENs, a single case had moderate intensity nuclear staining for Nkx2.2 with a staining extent of 2+.

Nuclear staining for Nkx2.2 was also observed in both cases of female reproductive tract NENs, with one case each of ovarian (Figure 5) and uterine origins, where the staining intensity was strong with an extent of 3+ in the former case and moderate with an extent of 2+ in the latter case.

Among the other NENs, the NEN in the salivary gland was negative for Nkx2.2. The NEN in the prostate was positive for Nkx2.2 with a moderate staining intensity and extent of 2+. Among the cases of NENs in the larynx, one

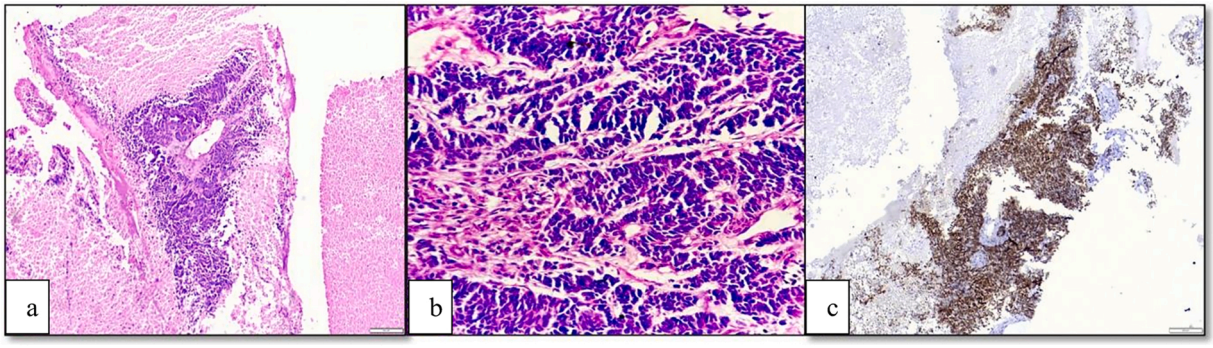


Figure 4: Nkx2.2 in pancreatic neuroendocrine neoplasm. (a) NEN of pancreas with large areas of necrosis (H and E, ×100). (b) Round tumor cells with high nuclear cytoplasmic ratio, hyperchromatic nuclei and scant cytoplasm in a cribriform pattern (H and E, ×400). (c) Nkx2.2 IHC showing diffuse(3+) and moderate intensity nuclear staining (DAB, ×200).

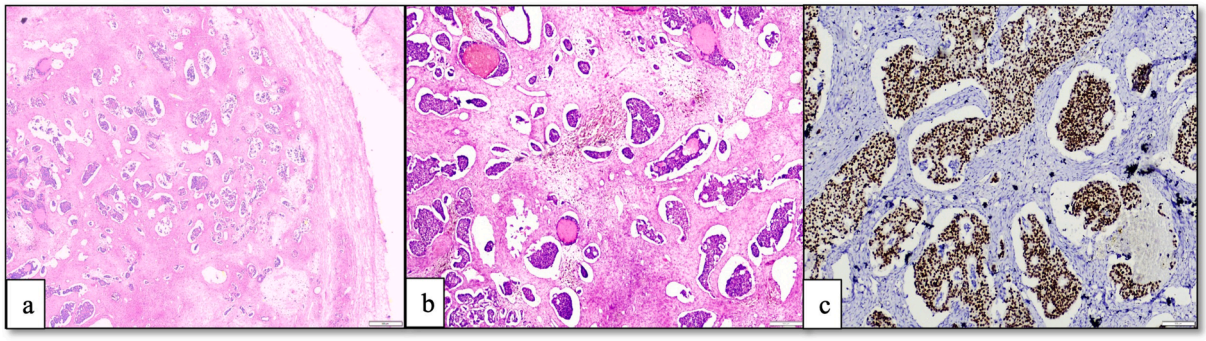


Figure 5: Nkx2.2 in ovarian neuroendocrine neoplasm (a) NEN ovary (H and E, ×20); (b) Tumor cells arranged in distinct lobules and nests separated by fibrous septa (H and E, ×100) (c) Nkx2.2 IHC showing diffuse (3+) and strong intensity nuclear staining (DAB, ×200).

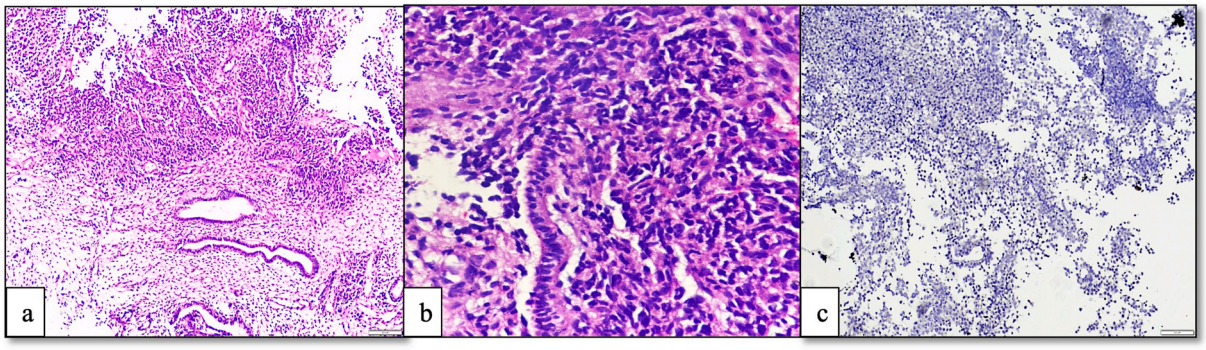


Figure 6: Nkx2.2 in neuroendocrine neoplasm of lung. (a) NEN of lung (H and E, ×100). (b) Tumor cells are round with hyperchromatic nuclei, arranged in sheets (H and E, ×400). (c) Nkx2.2 IHC showing negative staining (DAB, ×200).

case was positive for Nkx2.2 with a moderate staining intensity and extent of 3+, and the other case was negative.

Five out of six cases of lung NENs were negative for Nkx2.2. A single case of small cell carcinoma of lung exhibited weak intensity staining with an extent of 1+ (Figure 6). Table 1 summarizes the expression of Nkx2.2 in NENs at different sites.

Nkx2.2 expression in other mesenchymal tumors

Four cases consisted of two cases each of synovial sarcoma and undifferentiated pleomorphic sarcoma. Among these four cases, Nkx2.2 IHC positivity was observed in a single case of undifferentiated pleomorphic sarcoma with a weak staining intensity and extent of 1+, and the other case was negative. Both cases of synovial sarcoma were negative for Nkx2.2 IHC.

Nkx2.2 expression in nonmesenchymal tumors

Among the nonmesenchymal mimics of ES (n = 5) were melanoma (n = 3) and T cell lymphoblastic lymphoma (n = 2). Overall, Nkx2.2 positivity was observed in 1/5 (20%) cases.

A single case of melanoma exhibited weak intensity staining with an extent of 1+, whereas the other had negative staining. Both cases of T cell lymphoblastic lymphoma were negative for Nkx2.2 IHC.

Table 1: Nkx2.2 expression in NENs at different sites.

Site	Nkx2.2 Positive	Intensity	2+/3+ Staining	1+ Staining
Gastrointestinal	9/14	Strong (4/9; 44.4%) Moderate (3/9; 33.3%) Weak (2/9; 22.2%)	8/9 (88.8%)	1/9 (11.1%)
Pancreas	3/4	Strong (1/3; 33.3%) Moderate (2/3; 66.6%)	3/3 (100%)	–
Lung	1/6	Weak (1/6; 16.6%)	–	1/1 (100%)
Female genital tract	2/2	Strong (1/2; 50%) Moderate (1/2; 50%)	2/2 (100%)	–
Central nervous system	1/4	Moderate (1/1; 100%)	1/1 (100%)	–
Salivary gland	0/1	–	–	–
Prostate	1/1	Moderate (1/1; 100%)	1/1 (100%)	–
Larynx	1/2	Moderate (1/1; 100%)	1/1 (100%)	–

Table 2: Nkx2.2 expression in ES and its histomorphological mimics.

Tumor	Nkx2.2 positive	3+ staining	2+ staining	1+ staining
ES	15/17 (88.2%)	11/17 (64.7%)	4/17 (23.5%)	0/17
NEN	18/34 (52.9%)	10/34 (29.4%)	6/34 (17.6%)	2/34 (5.8%)
Mesenchymal				
1. Synovial sarcoma	0/2	–	–	–
2. Pleomorphic sarcoma	1/2 (50%)	–	–	1/2 (50%)
Nonmesenchymal				
1. Lymphoblastic lymphoma	0/2 (0%)	–	–	–
2. Melanoma	1/3 (33.3%)	–	–	1/3 (33.3%)

ES: Ewing's sarcoma; NEN: neuroendocrine neoplasm.

Table 3: Distribution of cases in different studies.

Final diagnosis	Wang et al. (2010)	Hung et al. (2016)	Goldman et al. (2018)	Yang et al. (2018)	Present Study
ES	–	40 (14.8%)	10 (9.3%)	–	17 (28.3%)
NEN	39 (100%)	–	20 (18.6%)	109 (100%)	34 (56.6%)
Mesenchymal					4 (6.6%)
1. Synovial sarcoma	–	10 (3.7%)	12 (11.2%)	–	2 (3.3%)
2. Pleomorphic sarcoma	–	–	–	–	2 (3.3%)
Nonmesenchymal			32 (29.9%)		5 (8.3%)
1. Lymphoblastic lymphoma	–	10 (3.7%)	11 (10.2%)	–	2 (3.3%)
2. Melanoma	–	20 (7.4%)	11 (10.2%)	–	3 (5%)
3. Small cell carcinoma	–	10 (3.7%)	10 (9.3%)	–	–

ES: Ewing's sarcoma; NEN: neuroendocrine tumor.

Immunohistochemistry for Nkx2.2 exhibited a high sensitivity of 88.2% and modest specificity of 53.4% in the diagnosis of ES. Nkx2.2 positivity was observed in various other tumor types, including NENs of GIT and pancreas origins. Infrequent Nkx2.2 positivity was also present in pleomorphic sarcoma (mesenchymal tumor) and melanoma (nonmesenchymal tumor). The overall positive predictive value of Nkx2.2 for ES was 0.5. The sensitivity of Nkx2.2 for NENs was 52.9% and the specificity was 34.6%. In addition, the sensitivities of Nkx2.2 for GIT and pancreatic origin NENs were as high as 66.6% and 76.1%, respectively. Table 2 summarizes the IHC expression of Nkx2.2 in ES and its histomorphological mimics.

Discussion

In the present study, we assessed Nkx2.2 IHC expression in ES, NENs, mesenchymal, and other nonmesenchymal tumors and compared the results with previous findings, as shown in Table 3.

The sample size (n = 60) in this hospital-based study does not reflect the true incidence of ES, NENs, mesenchymal, and nonmesenchymal tumors in the community.

ES is characterized by the presence of *EWSR1-FLI1* translocation, which results in fusion of the EWSR1 gene on chromosome 22 with the *FLI1* gene on chromosome 11. This translocation plays a crucial role in the pathogenesis of the disease by acting as an aberrant transcription factor that drives oncogenesis. Nkx2.2 is upregulated in response to this fusion protein. The expression of Nkx2.2 is a marker for ES but also a reflection of the underlying genetic alterations that drive the disease, making it a valuable tool for understanding the biology of ES.[4,5]

In our study, 88.2% of cases (15/17) were positive for Nkx2.2. Among these cases, 14 (82.3%) had strong or moderate staining intensities and 88.2% exhibited diffuse staining extents (3+/2+). Similarly, Hung et al. and Goldman et al. reported diffuse immunopositivity for Nkx2.2 in 93% of ES cases (37/40) and 100% of ES cases (10/10), respectively.[4,5]

We demonstrated the significantly high sensitivity (88.2%) of Nkx2.2 in the diagnosis of ES in a similar manner to that observed in previous studies.[4,5] However, the specificity was only 53.4% and lower compared with other studies.[4,5] The reduced specificity found in our study could have been due to the smaller sample size leading to a higher false-positive rate. In addition, the positive predictive value was 0.5 in our study and similar to that reported by Goldman et al., suggesting that although Nkx2.2 is a sensitive marker for ES, its use as a definitive diagnostic tool may be limited, thereby necessitating the use of additional markers for confirmation.[5]

NENs are a diverse group of tumors that originate from neuroendocrine cells, and their behavior is influenced by various genetic alterations. The Nkx2.2 gene plays a critical role in the development of neuroendocrine cells, and its expression in NENs likely reflects its importance in the pathogenesis of these tumors.[1]

Among 34 cases of NENs at different sites, 52.9% (18/34) were nuclear positive for Nkx2.2.

Among the 18 NENs cases (14 GIT cases and four pancreatic cases) included in this study, three cases were NETs and 15 were NECs. Overall, as high as 66.6% of cases (12/18) were positive for Nkx2.2, where 91.6% of cases (11/12) exhibited diffuse staining (3+/2+) and the staining intensity was strong or moderate for 83.3% of cases (10/12). These findings are consistent with previous studies by Yang et al., Goldman et al., and Wang et al.[13] who observed very high Nkx2.2 positivity in cases of well differentiated GI NETs, i.e., 100% (5/5; strong/moderate) (1), 86.4% (70/81),[5] and 92.3% (24/26)[13] of cases, respectively. Yang MX et al. found Nkx2.2 positivity in 70% of cases (12/17) of pancreatic NETs.[1] These findings are consistent with previous studies that demonstrating the presence of Nkx2.2 positivity in NENs of GIT origin, agreeing with the demonstrated role of Nkx2.2 in GIT tract endocrine cell differentiation.[1,5,13]

However, five out of six (83.3%) cases of primary NENs in the lung were negative for Nkx2.2, supporting previous studies that showed a lack of Nkx2.2 expression in NENs with lung origins.[1,13] Goldman et al. also demonstrated weak to moderate Nkx2.2 staining with extents of 1+ to 3+ in 26.7% of cases (4/15) of primary NENs in the lung.[5] The conspicuous absence of Nkx2.2 in lung NENs might be related to different developmental pathways that do not involve Nkx2.2, highlighting the marker's potential utility in distinguishing between different types of NENs based on their site of origin.

In the present study, we also observed Nkx2.2 positivity in NENs at other rare sites that have not been described in previous studies to the best of our knowledge, including a case of spinal NEN with moderate intensity staining and an

extent of 2+, two cases of female genital tract (uterus and ovary) NENs with strong intensity staining and extents of 2+/3+, a single case of prostate NEN with moderate intensity staining and an extent of 2+, and a single case of larynx NEN with moderate intensity and an extent of 3+.

Overall, we observed that Nkx2.2 had a sensitivity of 52.9% for NENs at different sites but the specificity was only 34.6%. The sensitivity of Nkx2.2 for GI and pancreatic NENs was 66.6%, and lower compared with that of 84% observed by Yang et al.[1]

In summary, Nkx2.2 exhibited higher sensitivity for GI and pancreatic origin NENs compared with NETs of lung origin, reinforcing the marker's potential utility for these specific tumor types.

Two cases each of synovial sarcoma and pleomorphic sarcoma were included in this study, where the single case of pleomorphic sarcoma exhibited weak intensity staining with an extent of 1+.

Hung et al. and Goldman et al. demonstrated positivity for Nkx2.2 in cases of synovial sarcoma,[4,5] where the former observed moderate staining in a single case of poorly differentiated synovial sarcoma and weak staining in 58% cases with an extent of 2+, and the latter observed diffuse staining in 33.3% cases with an extent of 2+. Therefore, Nkx2.2 has low sensitivity and specificity for synovial sarcoma and pleomorphic sarcoma according to previous studies.[4,5] The sporadic and weak expression highlights the need for additional markers to aid the accurate diagnosis of these sarcomas.

Among the three cases of melanomas and two cases of T cell lymphoblastic lymphomas included in this study, only one case of melanoma had weak intensity staining with an extent of 1+, whereas the remaining cases were negative for Nkx2.2 expression, confirming the limited expression of this marker in nonmesenchymal tumors, consistent with previous studies.[4,5] Therefore, studies do not support the routine use of Nkx2.2 in diagnosing these tumors, and thus the role of the marker remains minimal in these contexts.

Conclusion

In this study, we confirmed the utility of Nkx2.2 as a sensitive marker for a diverse set of tumors, highlighting the strengths and limitations of this marker. The sensitivity of Nkx2.2 was high for diagnosing ES and certain NENs (especially GIT and pancreatic origins), but its lower specificity and varied expression across other tumor types highlight the need for a cautious and context-specific approach as well as the employment of additional IHC markers when used in clinical practice. Our findings also emphasize the importance of understanding the genetic context in which Nkx2.2 operates, which can provide insights into its role in tumor biology and aid the development of more targeted diagnostic and therapeutic strategies. Our novel findings regarding the expression of Nkx2.2 in rare NENs warrants further investigation and may lead to new diagnostic applications and a better understanding of these tumors. Further research with larger sample sizes is needed to validate these findings and explore the potential use of Nkx2.2 in other tumor types.

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Conflict of interest

The authors have no conflict of interest to declare

Ethical approval

Institutional Ethical Committee approval was obtained (IEC no: SAIMS/IEC/196/2021).

Authors contributions

Conceptualization: SK. Data curation: MJ. Formal analysis: MJ, SK. Funding acquisition: none. Investigation: MJ, SK. Methodology: MJ, SK, AV, NM. Project administration: AV,NM. Resources: AV. Software: AV. Supervision: AV, NM. Validation: AV, NM. Visualization: SK, AV, NM. Writing − original draft: MJ, SK. Writing − review & editing: SK, AV, NM. Approval of final manuscript: all authors. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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