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ANATOMICAL PATHOLOGY

Expression of neuroendocrine markers in non-neuroendocrine endometrial carcinomas

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Summary

Neuroendocrine (NE) tumours are uncommon in the gynecological tract. In addition to their histological features, what defines NE carcinoma is the expression of markers such as chromogranin, synaptophysin and neural cell adhesion molecule (CD56) by immunohistochemistry (IHC). Although limited data have demonstrated that some high-grade uterine tumours may focally express these markers, the incidence of such labelling in endometrial carcinomas in general is not well known. The goal of this study was to characterise the expression of NE markers in a cohort of endometrial carcinomas. We searched our institutional surgical pathology database for hysterectomy specimens containing endometrial carcinomas. Cases demonstrating classic morphological features of NE carcinomas were excluded. IHC for synaptophysin, chromogranin and CD56 was performed in whole-tissue sections of formalin-fixed, paraffin-embedded (FFPE) tumours. Thyroid transcription factor 1 (TTF-1) was also included, given its positivity in a subset of small cell carcinomas. Marker expression was graded based on percentage of positive tumour cells (0, not detected; 1, 1–25%; 2, 25–50%; 3, >50%). Chi-square was used for statistical analysis and significance was set at $p<0.05$. In total, 71 carcinomas of endometrioid (EMCA; 26 cases), serous (20), clear cell (12), undifferentiated (2) and dedifferentiated (1) histologies were obtained, as well as 10 carcinosarcomas. The majority expressed one or more NE markers (47/71; 66%), with most positive cases showing focal (1+) staining of a single marker. Significantly more tumours stained positive for CD56 than synaptophysin (58% vs 7%, $p<0.01$). Clear cell carcinomas were the least likely to express any NE marker (4/12; 33%), whereas serous carcinomas (80%) and carcinosarcomas (100%) were the most likely. CD56 labelling was seen in 9/10 carcinosarcomas, in both epithelial (7/9) and mesenchymal (5/9) elements. A slightly greater proportion of non-endometrioid histological types stained positive for TTF-1 compared with endometrioid type (31% vs 12%, $p=0.06$). Immunohistochemical expression of NE markers is relatively common in endometrial carcinomas that lack classic NE histology. The most frequent pattern encountered in our study was focal (1–25%) labelling of a single marker. Synaptophysin appeared reliably negative, while CD56 was commonly present in non-NE histology. Clear cell carcinomas tend to be consistently negative, whereas carcinosarcomas and serous carcinomas frequently express at least one marker. Awareness of these data may help to avoid misdiagnosis of a neuroendocrine carcinoma in limited samples.

Key words: Neuroendocrine; endometrial carcinoma; synaptophysin; chromogranin; CD56; TTF-1.

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INTRODUCTION

Neuroendocrine (NE) tumours of the endometrium are uncommon neoplasms that range from low grade, well differentiated tumours (carcinoid) to high grade carcinomas with poor prognosis, such as small cell and large cell NE carcinomas.[1] Despite limited data, available evidence suggests that these high grade NE tumours may also exhibit aggressive behaviour, similar to other organ systems.[1,2]

The approach to diagnosis of NE tumours is largely based on haematoxylin and eosin (H&E) microscopy. Classic features of small cell neuroendocrine carcinomas include diffuse growth of round, poorly cohesive cells with condensed chromatin and scant cytoplasm, showing necrosis, brisk mitotic activity and crushing artifact.[3] Large cell neuroendocrine carcinomas exhibit, at least focally, well demarcated nests, trabeculae, or cords, with peripheral palisading, containing large polygonal cells that have vesicular or hyperchromatic nuclei, abundant cytoplasm and prominent nucleoli.[3]

Immunohistochemical markers to confirm NE differentiation include staining for chromogranin-A (here referred to as chromogranin only), synaptophysin, and neural cell adhesion molecule (CD56).[4] With respect to the expression of these IHC stains in endometrial NE tumours, the World Health Organization (WHO) criteria requires >10% of tumour cells to express any one of the aforementioned NE markers to establish the diagnosis of large cell neuroendocrine carcinoma, with the caveat that CD56 may not be as specific.[3] Other studies have recommended expression of two or more NE markers, or that >20% of tumour cells should be positive in this setting.[5,6] Finally, expression of NE stains in small cell neuroendocrine carcinoma is supportive, but not required to establish the diagnosis.

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In addition to the aforementioned NE markers, pulmonary NE tumours consistently stain for thyroid transcription factor (TTF-1), including more than 90% of small cell neuroendocrine carcinomas.[7] However, many NE carcinomas outside of the lung can express TTF-1. One study found that seven of 16 (43%) non-pulmonary small cell neuroendocrine carcinomas were positive for TTF-1, along with 100% (8/8) medullary thyroid carcinomas.[8] In the endometrium, TTF-1 expression appears to be non-specific, as one study found expression in six of 32 (18%) cases of endometrial carcinomas of endometrioid type (EMCA), and in three of 13 (23%) endometrial serous carcinomas.[9]

There are very limited published data regarding the presence, distribution and intensity of NE markers in endometrial carcinomas without 'classic' NE histology. Some of these studies were hindered by small sample sizes of poorly and/or undifferentiated endometrial carcinomas, or only evaluated a limited number of NE markers or histological types.[5,9–15] Description of the staining properties of NE markers in serous and clear cell carcinomas, in particular, is lacking.

The aim of this study was to examine the presence, distribution and intensity of NE markers (as well as TTF-1) in a large group of endometrial carcinomas of various grades and histological subtypes, but without histological evidence of NE differentiation.

MATERIALS AND METHODS

Following the Institutional Review Board approval, our surgical pathology database was searched for hysterectomy specimens spanning an 8-year period (2010–2017) with the diagnosis of endometrial carcinoma. A variety of histological subtypes, grades, and stages was selected randomly with the goal of obtaining a diverse number of tumours.

Slides were re-reviewed by two pathologists (AP, AM) utilising diagnostic criteria from the 2014 WHO Classification.[3] Pathological staging was evaluated at the time of diagnosis using the 7th edition of the College of American Pathology (CAP) approved American Joint Committee on Cancer (AJCC) standards.[16] If applicable, supplemental IHC was performed to re-classify, or confirm, the original diagnosis. Cases that were determined to show classic features of NE carcinoma were excluded from the study.

IHC for synaptophysin, chromogranin, CD56, and TTF-1 was performed on 4 µm thick whole sections of a selected block of formalin-fixed, paraffin-embedded (FFPE) tissue. The tumours were stained with a standard protocol using a Leica automated Bond System (Leica Biosystems, USA). Antibody dilutions were supplied from the manufacturer [ready to use (RTU); Leica] for each antibody clone: TTF-1 (SPT24), chromogranin (5HT), synaptophysin (27G12), and CD56 (CD564). Antigen retrieval for TTF-1 and synaptophysin was accomplished at pH 9.0 with incubation times of 20 and 15 min, respectively. Antigen retrieval for chromogranin and CD56 was accomplished at pH 6.0 for 15 min. Blocks that contained the largest quantity of tumour tissue were selected for staining. Marker expression was graded based on the percentage of positive tumour cells (0, no detectable expression; 1, staining of 1–25% of tumour cells; 2, 25–50%; 3, >50%) and intensity (0, no detectable staining; 1, faint staining; 2, moderate staining; 3, strong staining).

Statistical analyses were done using Stata IC 14 (StataCorp, USA). Proportional comparisons of staining were carried out using Fisher's exact test. All tests were two-sided, with significance set at $p<0.05$.

RESULTS

A total of 26 EMCA [12 International Federation of Gynecology and Obstetrics (FIGO) grade 1, six FIGO grade 2, and eight FIGO grade 3)], 20 serous carcinomas, 12 clear cell carcinomas, two undifferentiated carcinomas, and one dedifferentiated carcinoma were included in the study, along with 10 carcinosarcomas. The ages of the patients at diagnosis ranged from 31 to 87 years (mean 63.9). The mean tumour size was 52.9 mm (range 20–135 mm). Basic demographic information of patients in the cohort is shown in Table 1.

Overall, 47 of 71 cases (66%) expressed one or more of the NE markers (synaptophysin, chromogranin and CD56), frequently in a focal distribution (Tables 2 and 3). The number of cases expressing any one of these three NE stains was 32/71 (45%); while 14/71 (20%) expressed any two NE markers. There were 2/71 cases (3%) which stained positive for all three, at least focally.

There were only two cases (3%) that exhibited TTF-1 staining alone, while nine cases (13%) showed a combination of TTF-1 and CD56 expression, two cases (3%) showed staining of TTF-1 with CD56 and chromogranin, two cases (3%) showed TTF-1 with CD56 and synaptophysin, and two cases (3%) showed staining for TTF-1, CD56, chromogranin, and synaptophysin. These cases made a total of 17/71 tumours.

In regards to histological type, all carcinosarcomas (n=10), undifferentiated carcinomas (n=2) and dedifferentiated carcinoma (n=1) expressed at least one NE marker, followed by 16/20 serous carcinomas (80%) and 17/26 EMCA (65%). Clear cell carcinomas were the least likely to show any expression of any marker, seen only in 3/12 cases (25%) (Table 2).

Overall, the most common NE marker expressed was CD56, present in 41 cases (58%), followed by chromogranin in 20 cases (28%), TTF-1 in 17 cases (24%) and synaptophysin in five cases (7%).

When considering CD56, 17/41 positive cases (41%) demonstrated more than focal expression (>25% of tumour cells stained/2–3+). Focal expression of CD56 appeared slightly more frequent in non-EMCA histological types, including six serous carcinomas, six carcinosarcomas, one clear cell carcinoma, and one undifferentiated carcinoma. Three EMCA histological types showed focal expression of CD56 including one FIGO-1 and two FIGO-3 (Tables 3 and 4). Overall, non-EMCA did show slightly more frequent staining of CD56 (14 non-EMCA of 17 total; 82%), but without reaching statistical significance (p=0.09). In 9/10 carcinosarcomas that expressed CD56, 3/9 demonstrated expression in both sarcomatous and carcinomatous elements, 4/9 only in the carcinomatous elements, and 2/9 only in the sarcomatous elements (Fig. 1). The intensity of CD56 staining was the most variable of all stains tested (Table 5).

Chromogranin expression was observed in 20 cases including 12/26 cases (46%) of EMCA [four cases FIGO-1 (Fig. 2A,B), three cases FIGO-2, five cases FIGO-3], 5/18 serous carcinomas (28%), and 3/10 carcinosarcomas (on which the staining was restricted to the epithelial elements in all cases). Expression was more extensive (>25% of tumour cells/2+ or greater) in seven cases and, of these, five were of EMCA type and two were serous carcinomas (Table 4). Stain intensity in positive cases was moderate-to-strong in virtually all cases (19/20; 95%) (Table 5). Of the 12 FIGO grade 1 EMCA in this study, 4/12 (33%) showed staining for chromogranin, with three of these showing moderate or greater intensity stain in the majority of tumour cells.

Synaptophysin was the least frequently expressed NE marker (5 cases), and it was observed in three cases of carcinosarcoma, one clear cell carcinoma, and one FIGO grade 3 EMCA (Fig. 2C,D; Table 3). Only two cases demonstrated

Table 1 Case demographics, histological subtypes and pathological staging

Histological type	Mean age at diagnosis, years	Mean tumour size, cm	Pathological category and stage, *n*		
			T	N	M
EMCA (*n*=26)	60.6	4.4	T1a=10, T1b=10, T2=4, T3a=1, T3b=1	N0=18, N1=1, N2=2, NX=5	M(n/a)=26
SC (*n*=20)	64.5	5.3	T1a=7, T1b=4, T2=3, T3a=5, T3b=1	N0=9, N1=4, N2=3, NX=4	M1=1, M(n/a)=19
CCC (*n*=12)	65.7	5.0	T1a=4, T1b=3, T2=4, T3a=1	N0=6, N1=4, N2=2	M(n/a)=12
CS (*n*=10)	69.3	7.3	T1a=3, T1b=4, T2=2, T3a=1	N0=7, N2=1, NX=2	M1=1, M(n/a)=9
UC (*n*=2)	65.0	4.5	T1b=1[a]	N0=1[a]	M(n/a)=1[a]
DDC (*n*=1)	57.0	8.3	T3a=1	N0=1	M1=1
Aggregate data (*n*=71)	63.9	Mean 5.2 Median 4.5	T1a= 24, T1b=22, T2=13, T3a=9, T3b=2	N0=42, N1=9, N2=8, NX=11	M1=3, M(n/a)=67

CCC, clear cell carcinoma; CS, carcinosarcoma; DDC, dedifferentiated carcinoma; EMCA, endometrioid carcinoma; SC, serous carcinoma; UC, undifferentiated carcinoma.
[a] Data unavailable for one case with respect to that particular data element.

Table 2 Endometrial carcinomas immunohistochemically positive for one or more NE markers (including TTF-1)[a]

Histological type	Positive cases/total
EMCA FIGO-1	7/12 (58%)
EMCA FIGO-2	4/6 (67%)
EMCA FIGO-3	6/8 (75%)
SC	16[a]/20 (80%)
CCC	3[a]/12 (25%)
CS	10/10 (100%)
UC	2/2 (100%)
DDC	1/1 (100%)
Total	49[a]/71 (69%)

CCC, clear cell carcinoma; CS, carcinosarcoma; DDC, dedifferentiated carcinoma; NE, neuroendocrine; SC, serous carcinoma; UC, undifferentiated carcinoma.
[a] 2/49 cases showed isolated TTF-1 staining (one CCC and one SC carcinoma); the remaining cases expressed TTF-1 combined with one or more other NE markers.

Table 3 Patterns of expression of NE markers

Marker	Focal staining,[a] positive cases/total	More than focal staining,[a] positive cases/total
Synaptophysin	5/71	2/71
TTF-1	17/71	4/71
Chromogranin	20/71	7/71
CD56	41/71	17/71

NE, neuroendocrine.
[a] Focal staining denotes staining in 1–24% of tumour cells (1+). More than focal staining denotes staining in 25%+ tumour cells (2–3+).

more than focal staining, and both of these tumours were carcinosarcomas (Table 4). Moderately intense staining was the most frequent pattern seen (Table 5).

Among the 17 cases that expressed TTF-1, staining distribution was predominantly focal (13/17 cases; 76%) but the intensity of staining was moderate-to-strong in virtually all cases (16/17 cases; 94%) (Tables 3–5). A greater proportion of non-EMCA histologies expressed TTF-1 when compared with EMCA (14/17 TTF-1 positive cases were non-EMCA; 82%) including six serous carcinomas, four carcinosarcomas, three clear cell carcinoma (Fig. 3), and one undifferentiated carcinoma, although this difference did not reach statistical significance (p=0.09).

After excluding TTF-1, 16/71 (22%) cases showed expression of two or more of any NE markers. Synaptophysin was expressed with another NE marker in all but one case, and of the 20 cases staining for chromogranin, the majority of cases (14/20; 70%) stained with an additional marker of CD56 and/or synaptophysin.

DISCUSSION

While the National Comprehensive Cancer Network provides recommendations for the treatment of high-risk endometrial cancer, there are no specific recommendations for the management of NE carcinoma.[17] However, as NE tumours of other disease sites are treated with chemotherapeutic agents which are not standardly used for other endometrial histologies, differentiating these tumours from other types of endometrial carcinomas is crucial for directing care.

The IHC expression of NE markers in 'conventional' endometrial carcinomas is relatively frequent. However, we observed that the simultaneous expression of two or more NE markers, even focally, is less common (20%). This would support utilising more than one marker in the diagnosis of NE

Table 4 Number of cases based on histological type with more than focal staining (2+/3+) of NE markers

Marker	FIGO 1 EMCA	FIGO 2 EMCA	FIGO 3 EMCA	SC	CCC	CS	UC	Total
CD56	1	0	2	6	1	6	1	17
Chromogranin	3	1	1	2	0	0	0	7
TTF-1	1	0	0	1	1	0	1	4
Synaptophysin	0	0	0	0	0	2	0	2

CCC, clear cell carcinoma; CS, carcinosarcoma; EMCA, endometrioid carcinoma; FIGO, International Federation of Gynecology and Obstetrics; SC, serous carcinoma; UC, undifferentiated carcinoma.

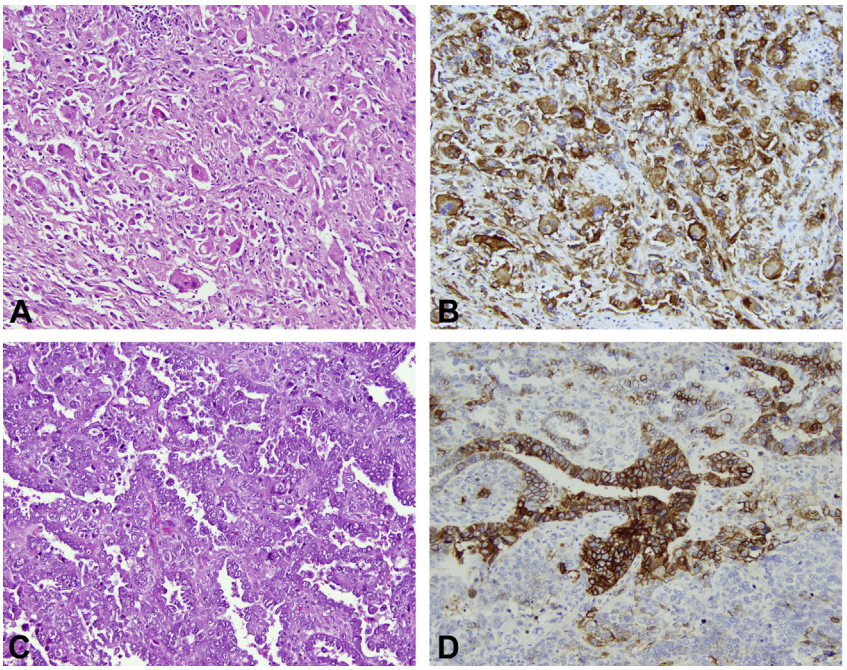

Fig. 1 Example of a carcinosarcoma case on which the sarcomatous component (A,B) stained strong and diffusely for CD56. The carcinomatous areas in this tumour (C,D) were also positive for CD56, mostly in a patchy distribution. (A,C: H&E; B,D: immunohistochemistry.)

Table 5 Intensity of staining in positive cases, based on each immunohistochemistry

Antibody	1+ (faint)	2+ (moderate)	3+ (strong)	Total positive
CD56	13	16	12	41
Chromogranin	1	13	6	20
TTF-1	1	6	10	17
Synaptophysin	0	4	1	5

tumours when such a diagnosis is being considered, as currently recommended by some authors.[5,6] Chromogranin with co-expression of synaptophysin, in particular, was rare. In regards to solo positivity, diffuse synaptophysin positivity was seen less frequently (2/71) than chromogranin (7/71) or CD56 (17/71), suggesting that synaptophysin may show less 'aberrant' staining of non-EMCA subtypes than chromogranin, in this setting.

The non-specificity of CD56 observed in this study limits its usefulness in the assessment of true NE differentiation, particularly if it is the only IHC evidence. Additionally, CD56 demonstrated the most variable intensity, with the largest amount of weakly positive cases, thus making stain interpretation difficult. Synaptophysin and chromogranin staining often showed moderate to strong intensity. Staining for CD56 was seen in a large subset of cases, which reinforces the limited utility of this stain as frontline or as the sole definitive evidence of NE differentiation.

It is important to recognise that CD56 belongs to the immunoglobulin family of cell surface adhesion proteins, and is expressed in neural, neuroectodermal, and neuroendocrine tissues, but may also be seen in haematolymphoid cells and neoplasms, skeletal muscle tissue, and other tumours.[18] In contrast, synaptophysin is directly involved with synaptic transmission, and chromogranin is a major component of secretory granules, both of which intuitively lead to a higher degree of specificity when expressed, and of which staining was decidedly less common in our series.

Alkushi and colleagues found 11/52 (21%) EMCA (amongst FIGO grades 1 and 3) and 3/23 (13%) serous carcinomas to express synaptophysin,[19] which was somewhat higher than the level of expression seen in our study. In a study of 47 carcinosarcomas, eight total cases were positive for either chromogranin or synaptophysin and 21/46 cases showed expression of CD56 (of approximately the same distribution of staining in the carcinomatous and sarcomatous areas).[10] We found a similarly common incidence of CD56 positivity (6/10 cases), but in contrast, the carcinosarcomas in our study were more commonly synaptophysin and chromogranin positive (three cases each). This difference may be due to the differences in criteria as we considered cases with a low level of expression as positive, whereas the aforementioned study commented that most NE marker staining, when observed, was 'multifocal'.

TTF-1 staining was seen in 24% of cases (17/71 cases), and almost always in a focal pattern. Our distribution of staining seems consistent with previous research from Siami and colleagues who demonstrated that approximately 19% endometrial EMCA and 23% of serous carcinomas stained for TTF-1, mostly in a patchy fashion.[9] We also observed that TTF-1 stained a slightly greater proportion of non-EMCA types compared with EMCA histology (31% of non-EMCA

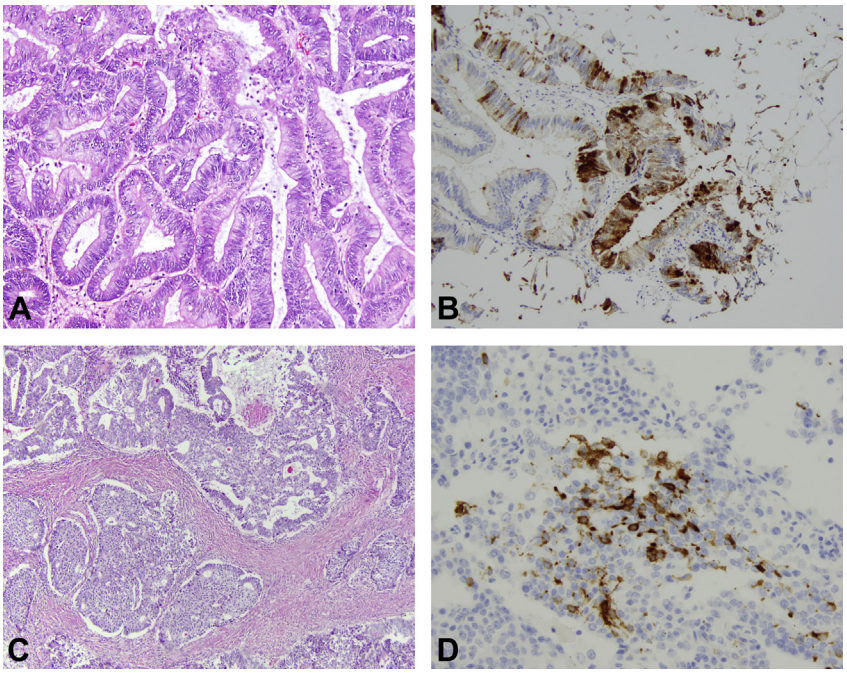

Fig. 2 Examples of two cases of endometrioid adenocarcinoma. (A) Well differentiated tumour (FIGO 1) showing patchy positivity for chromogranin (B). (C) FIGO2 endometrioid carcinoma with patchy staining for synaptophysin (D). (A,C: H&E; B,D: immunohistochemistry.)

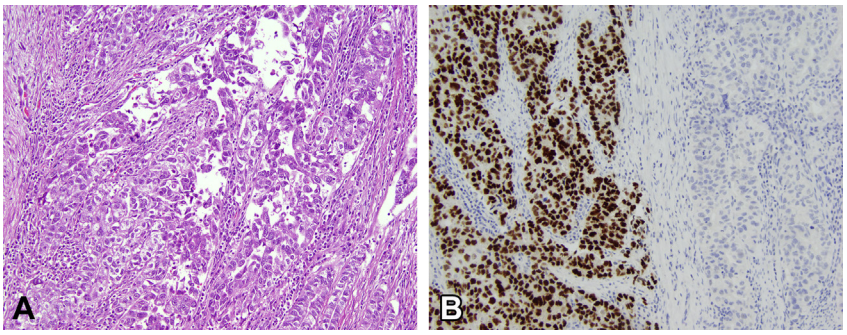

Fig. 3 Low power view of a endometrial clear cell with focal solid growth and positive, heterogeneous nuclear staining for TTF-1. (A: H&E; B: immunohistochemistry.)

types vs 12% of EMCA types, p=0.06). Interestingly, TTF-1 was frequently (with exception of two cases) positive in tumours co-expressing CD56 (15 cases).

Overall, we observed that focal staining for TTF-1 can be seen with moderate frequency, and would need to be taken into account when considering tumours which are commonly TTF-1 positive, including mesonephric carcinomas and metastatic lung carcinomas. Of note, a prior study has shown that TTF-1 is rarely expressed in primary NE carcinomas of the endometrium.[1] Therefore, it seems that TTF-1 stain has little diagnostic utility in confirming NE carcinomas in the uterus, and is probably more likely to be positive in other primary carcinomas of non-EMCA histology.

Perhaps the least available information in the literature is in regards to endometrial clear cell carcinoma and NE expression. We found clear cell carcinoma to be the least likely histological subtype to express any NE markers, with only two of 12 showing any expression, along with a third case that demonstrated only TTF-1 positivity. Moreover, of these three cases, one had only focal expression of TTF-1, and the other had focal expression of TTF-1 and CD56.

As stated previously, carcinosarcomas in our study exhibited the greatest degree of aberrant IHC (especially for CD56), which were similar results to previous studies.[10] In 1991, George and colleagues reported 8/47 (17%) carcinosarcomas had evidence of NE 'differentiation' by synaptophysin or chromogranin staining, and appeared to have a worse prognosis with a more rapid fatal course, although this finding was only seen in a few cases and could not be statistically substantiated.[10]

Although we evaluated few cases of undifferentiated/dedifferentiated carcinoma, we found only focal staining, which has been documented previously.[14,20]

The cause of NE marker expression in 'conventional' endometrial tumours remains unclear, but appears to be rather common. There have been very few studies that have attempted to identify any putative cell of origin or explain the relative rarity of NE tumours in this anatomical location. A recent and detailed human study which examined both eutopic endometrium and endometriotic tissues found that endometrial tissues contained small dispersed synaptophysin positive epithelial cells, predominantly within the secretory

phase, and that these cells were significantly more common in those with endometriosis.[21] Another possibility for the origin of NE expression could be some degree of trans-differentiation of one particular histological subtype into cells with a NE morphology and potentially an immunophenotype differing from the original histomorphology. Such a concept is perhaps more favourable, given the occasionally encountered NE carcinoma in combination with other histological subtypes, including EMCA and serous carcinomas.[2,13,22–25]

Based on the frequent expression of NE stains in many non-small or large cell NE carcinomas, the use of any cut-off to clearly delineate a diagnosis we find to be of arbitrary value unless there is further literature to support clear predictive or prognostic advantage. Rather, the diagnostic decision should largely rest on the backbone of H&E morphology that focuses on clear-cut architectural and cytological evidence of NE differentiation. In limited biopsy samples, one should avoid the utilisation of NE IHC unless there is clear evidence of NE histology, as the potential positive results may not necessarily represent the true nature of these tumours.

One also needs to understand the loose terminology applied in pathology practice, on which the description of 'differentiation' and 'features' are often used to describe tumours that show evidence of NE IHC expression. As no specific criteria strictly define these terms, we tend to avoid these nomenclatures unless a clear component showing NE histology is present in a tumour that is otherwise 'conventional'. This is supported by the findings of our study, and also avoids confusion in the clinician's understanding on how to approach these tumours, as most likely this information will not be used for management.

The strengths of this study include the large variety of histological subtypes and grades, the number of NE markers investigated, and the more precise quantitation of NE marker staining in tumours lacking conventional NE morphological features. The biggest weakness is the small sample size of specific histological subtypes, which may have limited the power to detect differences in staining. Moving forward, the next steps are to evaluate the significance of NE marker expression in our cohort for diagnostic, prognostic, and therapeutic information. Also, new and promising markers, such as insulinoma-associated protein 1 (INSM1) appear to be on the horizon to assess for NE differentiation.[26] Should their use become more widespread, further validation with prior NE marker expression in endometrium may also be undertaken.

CONCLUSION

IHC expression of NE markers in tumours lacking carcinoid, small cell or large cell neuroendocrine morphologies was commonly observed in our study, mostly in a patchy pattern. This reinforces the importance of proper morphological characterisation before considering the diagnosis of a neuroendocrine tumour (or 'component') based solely on IHC.

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