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Utility of Alpha-methylacyl-CoA racemase (p504s) Immunohistochemistry In Distinguishing Endometrial Clear Cell Carcinomas from Serous and Endometrioid Carcinomas

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Abstract

The expression of alpha-methylacyl-coenzyme-A-racemase (AMACR) has previously been reported in 75 to 100% of urethral/bladder clear cell carcinomas, tumors that are known to display broad phenotypic overlap with their identically-named müllerian counterparts. Herein, we assess the utility of AMACR in distinguishing endometrial clear cell carcinomas (CCC) from endometrial serous carcinomas (ESC) and endometrial endometrioid carcinomas (EEC). 111 endometrial carcinomas in a tissue microarray, including 49 CCC, 13 ESC and 49 EEC, were assessed for AMACR immunoreactivity, with results scored semi-quantitatively (scores 0, 1+, 2+, 3+ for 0%, 1-5%, 6-50%, >50% immunoreactive cells respectively). 50 (45%) of the 111 carcinomas were AMACR-positive, with the following score distribution: CCC: 0 (n=12), 1+ (n=12), 2+ (n=3), 3+ (n=22); EEC: 0 (n=38), 1+ (n=4), 2+ (n=4), 3+ (n=3); ESC: 0 (n=11), 1+ (n=1), 2+ (n=0), 3+ (n=1). AMACR expression was significantly more frequent in CCC (75%) than in ESC (15%) or EEC (22%), $p<0.0001$. The sensitivity and specificity of AMACR expression in classifying a carcinoma as CCC were 0.75 (95% CI: 0.61-0.86) and 0.79 (95% CI: 0.66-0.88) respectively, with an odds ratio of 11.62 (95% CI: 5-28, $p < 0.001$), and an area under the curve of 0.79 (95% CI: 0.68 to 0.88). These findings indicate that AMACR expression is strongly associated with CCC and displays a relatively robust diagnostic test performance. However, its practical utility may be limited by the focal nature of its expression in 32% of the AMACR-positive CCC cases, as well as its expression in 15-22% of the non-CCC histotypes.

Keywords

Alpha-methylacyl-CoA-racemase; p504s; AMACR; immunohistochemistry; endometrial clear cell carcinoma

Introduction

Alpha-methylacyl-CoA racemase, also known as AMACR or p504s, is an evolutionarily conserved enzyme that plays a key role in the metabolism of branched-chain fatty acids, which are important components of human cells (1). AMACR was originally identified using cDNA library subtraction in conjunction with high throughput microarray screening of normal and cancerous prostate tissues (2). The anti-AMACR antibody soon thereafter found significant diagnostic application as an ostensibly sensitive and specific marker for prostatic adenocarcinoma (3,4), and this remains the most common application for this marker in routine practice. However, AMACR expression has now been reported in numerous extraprostatic neoplasms (5-7) as well as in benign prostatic processes (8), and its greatest diagnostic utility is now recognized to lie in narrowly tailored and specific diagnostic contexts. In the gynecologic tract, AMACR expression has been demonstrated in 0-7.4% of endometrial carcinomas and 0-11.8% of ovarian carcinomas (6,7,9), and may have some utility in distinguishing metastatic colorectal carcinoma from primary ovarian adenocarcinomas (10).

Recent studies have reported that 75 to 100% of clear cell carcinomas of the urinary bladder and urethra express AMACR (10-12). Since clear cell adenocarcinomas of the lower urinary tract are known to display broad morphologic and immunophenotypic overlap with clear cell adenocarcinomas of the gynecologic tract (14), we hypothesized that the latter also express AMACR. In this study, we assess the utility of AMACR immunohistochemistry in distinguishing clear cell carcinomas of the endometrium (CCC) from endometrial serous carcinomas (ESC) and endometrial endometrioid carcinomas (EEC).

Materials and Methods

A dataset comprised of 77 endometrial clear cell carcinomas (CCC) was originally contributed by 9 gynecologic pathologists from 9 US institutions in response to a request for cases whose morphologic features were felt to be clearly diagnostic of CCC (15). Subsequently, one slide from each case was independently reviewed by 3 gynecologic pathologists (VP, JH, OF), with instructions to classify each case into CCC or non-CCC (any cancer other than pure CCC). If all 3 reviewers agreed with the primary contributor's diagnosis of CCC, the case was moved into a “consensus group”, which served as a gold standard for assessing AMACR performance in the diagnosis of CCC. If any of the 3 reviewers classified a case as non-CCC, it was transferred to a “non-consensus group”. After this review, 60 cases were assigned to the consensus group and 17 to the non-consensus group. In the latter group, 9, 5 and 3 cases were classified as non-CCC by 3, 2 and 1 reviewer(s) respectively. The aforementioned review was performed in the context of a previous study (15), and this dataset has been used, in total or subsets, in previous studies from this group (15-19).

Tissue microarrays (TMAs) were constructed from the 54 cases in the consensus group for which paraffin embedded tissue blocks (or punches) were available, using a manual arrayer (Beecher Instruments, Sun Prairie, WI). Two separate arrays were constructed, each containing a 1.0 mm-diameter tissue core from every case. Also included in the TMAs were several other cancers and tissue types, including endometrial serous carcinoma (ESC, n=17), endometrial endometrioid carcinomas (EEC, n=49, comprised of 12, 19, 18 FIGO [International Federation of Gynecology and Obstetrics] grades 3, 2 and 1 cases respectively), secretory endometrium (n=10), proliferative endometrium (n=10), and atrophic endometrium (n=5). The EEC and ESC cases were comprised only of morphologically unambiguous examples of each entity, as selected by one author (OF) and verified by another (MMD). Only the TMA cases were used to assess AMACR test performance. However, 48 cases were also evaluated in whole tissue sections not only for comparison with the TMA, but to gain additional insights into AMACR staining patterns. In this group were 15 randomly selected cases from the TMAs that were evaluated on whole tissue sections to verify the staining patterns on the TMA. These 15 cases included 4 CCC, 6 EEC, 1 ESC, and 4 non-neoplastic endometria. Also included were 8 cases of non-CCC endometrial carcinomas with clear cells, evaluated to ascertain that the AMACR staining was not simply a reflection of cytoplasmic clarity. These 8 cases included 4 endometrioid carcinomas of the secretory type, 2 endometrioid carcinomas with clear cell change associated with squamous differentiation, 1 EEC with non-specific focal clear cell change, and 1 ESC with focal clear cells. Additionally, 8 cases of non-neoplastic endometrium composed of glands with notably clear cytoplasm were evaluated. Included in this group were 3 cases with foci of Arias-Stella reaction, 1 case with clear cell metaplasia, and 4 cases of secretory endometrium at the early to mid-luteal phase. All cases for which there was no diagnostic consensus were also evaluated on whole tissue sections.

Immunohistochemical analyses were performed on all cases on the Leica Bondmax platform (Leica Micro-systems, Buffalo Grove, IL, USA). Antigen retrieval was performed using the Bond Epitope Retrieval Solution 1. Slides were then incubated with a monoclonal antibody to AMACR (Dako, Carpinteria, CA; clone 13H4; Prediluted; 15 min), with the Bond polymer Refine detection system (Leica Microsystems) used for secondary antibody and visualization. The 13H4 clone is an antibody that was raised against the full-length recombinant AMACR protein. Cases were jointly scored by 2 authors (MMD, OF), with scores assigned on a 4-tiered scale: 0, 0% tumor epithelial cells positive; 1+, 1-5% positive; 2+, 6-50% positive; 3+, >50% positive.

In our calculations on AMACR test performance (sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratios) relative to the diagnosis of CCC, the consensus group was used as the gold standard, EEC and ESC were classified as a single group of "cancers other than CCC", and all non-neoplastic samples were excluded. In these analyses, AMACR-positive CCC were classified as true positives and AMACR-negative CCC were classified as false negatives; AMACR-positive EEC/ESC were classified as false positives and AMACR negative EEC/ESC as true negatives. Odds ratios were generated measuring the size of the predictive effect of AMACR expression with the consensus CCC histotype as outcome. A Wilcoxon statistic was used to generate an empirical estimate of the area under the receiver operating curve. In correlation analyses between the TMA and whole tissue sections, AMACR scores were dichotomized (0 versus 1+ to 3+ for "negative" versus "positive") and correlation coefficients were generated using a Pearson Product-Moment Correlation test. Potential relationships between AMACR expression and clinicopathologic variables or patient outcomes were assessed using Fisher's exact tests, 2-tailed student t tests, and Cox Proportional Hazards Survival Regression analyses. Analyses were performed using SPSS software version 19 (SPSS Inc., Chicago, IL), Microsoft Excel® (Microsoft Corp., Redmond, WA), and the VassarStats program (www.vassarstats.net). $p<0.05$ was considered to be statistically significant in all analyses. This study was approved by the Vanderbilt University Institutional review board.

Results

9 (7.5%) of the 120 carcinomas on the TMA were not scoreable due to the absence of lesional tissue on the analyzed section. 50 (45%) of the remaining 111 carcinomas were AMACR positive (≥1+ score). Staining localization was invariably cytoplasmic (Figures 1 and 2). Stromal staining, where present, was minimal and was easily discernable from epithelial cell staining. Myometrial and inflammatory cells were AMACR-negative, whereas scattered endothelial cells within the tumor expressed the protein. Proteinaceous secretions as well as zones of tumor necrosis were also frequently immunoreactive for AMACR. The distribution of AMACR scores is outlined in table 1. In the AMACR-positive CCC cases, there was a somewhat bimodal distribution, in that 34 (92%) of the 37 positive cases had a score of either 1+ (12/37) or 3+ (22/37). AMACR expression was significantly more frequent in CCC (75%) than ESC (15%, $p=0.00013$) or EEC (22%, $p<0.0001$). There was no significant difference between EEC and ESC regarding the frequency of AMACR expression (22% versus 15%, $p=0.71$). Using the aforementioned definitions, there were 37, 49, 12, and 13 true positives, true negatives, false negatives and false positives respectively. Accordingly, the sensitivity and specificity of AMACR expression in classifying a carcinoma as being of the clear cell histotype were 0.75 (95% confidence interval [CI]: 0.61-0.86) and 0.79 (95% CI: 0.66-0.88) respectively, with an odds ratio of 11.62 (95% CI: 5-28, $p < 0.0001$). Positive and negative predictive values were 0.74 (95% CI: 0.59-0.85) and 0.80 (95% CI: 0.68-0.89) respectively. Positive and negative likelihood ratios were 3.60 (95% CI: 2.16-5.99) and 0.31 (0.19-0.51) respectively. A Wilcoxon estimate of the area under the receiver operating curve was 0.793 (95% CI = 0.703 to 0.884). If the threshold for positivity was increased to an AMACR score of ≥2+, the sensitivity, specificity, positive and negative predictive values were 0.51 (95% CI: 0.37-0.65), 0.92 (0.81-0.97), 0.83 (95% CI: 0.65-0.94), and 0.69 (95% CI: 0.58-0.79) respectively. At the 3+ threshold, these values were 0.45 (95% CI: 0.31-0.60), 0.94 (0.84-0.98), 0.85 (95% CI: 0.64-0.95), and 0.68 (95% CI: 0.57-0.78) respectively. In the non-neoplastic endometrium group, 9 (37.5%) of 24 cases were AMACR positive, with a tendency for higher positivity rates in secretory endometrium (7 of 10) as compared to proliferative endometrium (2 of 7, $p=0.07$). The clinicopathologic features of our consensus group CCC patients in the TMA are summarized in table 2. In this group, AMACR expression was not significantly associated with clinicopathologic factors

on univariate analysis, including patient age, FIGO stage, myometrial invasion, necrosis, lymph node status, lymphovascular invasion, mitotic index, and predominant architectural pattern of tumor. In a multivariate model that also included patient age, FIGO stage, mitotic index, and predominant architectural pattern of tumor, AMACR status was not associated with overall survival at either the ≥1+ (correlation coefficient -0.4985; 95% CI -1.2705 to 0.2735, p=0.2056) or the 3+ (correlation coefficient -1.66; 95% CI -3.8196 to; p=0.51) score thresholds for positivity.

In the non-consensus group, 9 (53%) of 17 cases were AMACR positive, with 8, 4, 2 and 3 cases having scores of 0, 1+, 2+ and 3+ respectively (table 1, figure 3). 6 (75%) of the 8 cases with a score of 0 had been classified as non-CCC by all 3 reviewers; the remaining 2 cases had been classified as non-CCC by 2 of 3 reviewers. Similarly, 2 of the 4 cases with scores of 1+ had been classified as non-CCC by all 3 reviewers; the remaining 2 cases had been classified as non-CCC by 2 of 3 reviewers. For the 5 cases with scores of 2+ or 3+, 3, 1, and 1 case(s) had been classified as non-CCC by 1, 2, and 3 reviewers respectively. Therefore, 100% of cases with scores of 0 had been classified as non-CCC by at least 2 reviewers, whereas 60% of the cases with scores of 2+ or 3+ had been classified as non-CCC by only 1 reviewer.

In the other tissues tested in whole sections, 7 of 8 endometrial carcinomas with clear cells were AMACR negative (see table 1). The single AMACR positive case had a score of 1+, and was an EEC with clear cell change associated with squamous differentiation. However, the focus of immunoreactivity was in a conventional glandular area and not in an area with clear cells. 2 of 4 secretory endometria with notably clear cytoplasm showed 2+ AMACR scores; the other 2 were AMACR negative, as were 3 cases with foci of Arias-Stella reaction, and 1 case with clear cell metaplasia.

There was significant positive correlation between the TMAs and whole tissue sections in our TMA verification set, with a correlation coefficient of 0.78 (p=0.0033) at the 0 versus ≥1+ score threshold. However, 2 cases - both CCC - had scores of 0 on the TMAs, but 1+ and 2+ on the corresponding whole tissue sections.

Discussion

The histotyping of high grade endometrial carcinomas is recognized to be a clinically significant endeavor (20), as well as one that is potentially fraught with significant lack of interobserver reproducibility (19,21). Although most cases of CCC are readily diagnosed based on their distinctive composite of morphologic features, a subset may cause diagnostic difficulties because they display significant overlap in morphologic attributes with the other major histotypes of endometrial carcinoma. Included in this group are purely solid carcinomas with clear cells, adenocarcinomas whose only CCC-like features are clear cells, the presence of clear cell-rich, cytoarchitecturally distinct areas in otherwise typical cases of EEC or ESC, and other histotypically ambiguous carcinomas with large clear cell or hobnail populations (19-21). The traditional immunohistochemical approach to resolving differential diagnoses that include CCC has been to deploy multi-marker panels [including various combinations of p16, p53, insulin-like growth factor-II messenger RNA-binding protein 3, high-mobility group AT-hook 2, intestinal trefoil factor 3 (TFF3), hormonal receptors, and others (15,20,21)] that are primarily geared towards *excluding* EEC or ESC, since the latter 2 tumors are significantly more common than CCC, have been studied more extensively, and accordingly, have more specific and sensitive markers. However, antibody panels are recognized to have their greatest diagnostic value when they contain admixtures of markers that expected to be positive and those that are expected to be negative for each of the most likely differential diagnostic considerations.

Recently, two markers have been reported that display a relatively strong association with CCC and accordingly allow a putative CCC case to be confirmed by a positive, rather than a negative immunophenotype. Hepatocyte nuclear factor 1β (HNF-1β), the first of such markers, was originally identified through gene expression profiling analyses of ovarian clear cell carcinomas, and was found in subsequent validation studies to be highly sensitive and specific marker for the clear cell histotype among ovarian carcinomas (22). Similar findings were reported in a small study of 33 endometrial carcinomas, wherein all CCC were HNF-1β positive and all non-CCC were negative (23). Studies published subsequently, however, demonstrated at least focal HNF-1β immunoreactivity in 22-60% of ESC, 5-35% of EEC, in a wide variety of non-clear cell cervical carcinomas, and in only 67-78% of uterine clear cell carcinomas (16,24-26). More recently, we evaluated the utility of the aspartic peptidase “napsin A” in distinguishing CCC from ESC and EEC, and found this marker to be both a highly sensitive and highly specific marker for CCC (15).

In the present study, we evaluated AMACR as another potential immunohistohemical marker of the clear cell histotype. AMACR was found to be frequently expressed in CCC (75%) and to be occasionally expressed in ESC (15%) and EEC (22%). The sensitivity and specificity of AMACR expression in classifying a carcinoma as being of the clear cell histotype were found to be 0.75 and 0.79 respectively. These findings suggest that AMACR may indeed be a diagnostically useful biomarker of CCC. However, we also found that a potentially major drawback to the use of AMACR as a CCC-related marker is the frequent focality of its expression. 32% of our AMACR positive CCC showed immunoreactivity in only 1-5% of tumor cells, although it was generally reassuring that in two randomly selected 1 mm cores of the tumors - which is akin to a scenario that may be encountered in an endometrial biopsy - these areas of focal immunoreactivity were still captured. Nevertheless, if the threshold for positivity were increased to an AMACR score of ≥2+, the sensitivity of this marker for CCC decreased to 0.51 whereas specificity increased to 0.92.

Another potential limitation of AMACR is related to its general test performance, given that 15% of ESC and 22% of EEC were found to be AMACR positive. In our opinion, as a marker whose sensitivity and specificity are both in the 0.70 to 0.80 range, AMACR is a useful marker to support a CCC diagnosis, as well as one whose specificity and potential focality of expression are such that it should not be used in isolation for this purpose. Our current findings, in conjunction with our previously published data, indicates that among the three CCC-related markers, napsin A (15) (sensitivity 0.88; specificity 0.98) clearly outperforms AMACR (sensitivity 0.75; specificity 0.79), which in turn outperforms HNF-1β (16) (sensitivity 0.73; specificity 0.54). Since none of the putative CCC markers enjoy perfect sensitivity and specificity, a panel approach may ultimately allow a CCC diagnosis to be rendered with maximal confidence in a diagnostically challenging case. Parenthetically, we directly compared our current findings on AMACR with those from our previous study on napsin A (15), since both studies were performed on essentially identical datasets. Requiring positivity for *both* napsin A and AMACR for a CCC diagnosis resulted in a sensitivity and specificity of 0.71 and 0.99 respectively. In other words, the combined positive immunophenotype had a decreased sensitivity and increased specificity as compared with the individual phenotypes. However, if positivity for *either* marker is the standard when both are used in tandem, the sensitivity for CCC increased to 0.92 whereas specificity was still high at approximately 0.85. Perhaps more importantly, the negative predictive value in the first scenario was over 0.90. Thus, negativity for *both* napsin A and AMACR is highly reassuring for a non-CCC diagnosis, and we recommend that both markers be included in the panel if CCC is a plausible diagnostic consideration. By combining the current findings with those in the recent literature, a useful diagnostic panel emerge which can be modified based on the specific diagnostic scenario and antibody availability. The most useful combinations include the ER+/PR+/TFF3+/IMP3- profile in

most grade 3 EEC that distinguishes them from CCC and ESC (27), the ER-/p53+/p16+ profile in most ESC that distinguishes them from grade 3 EEC (24), and the napsin A+/AMACR+ profile in most CCC that distinguishes them from EEC and ESC (15).

The high frequency with which CCC expresses AMACR is noteworthy, and is rivaled only by papillary renal carcinomas, prostatic adenocarcinomas, and colorectal carcinomas. This finding raises the possibility of applications outside of the diagnostic realm, including its potential as a target for directed therapy, or as a prognostic marker. *In vitro* studies have demonstrated that the attenuation of AMACR expression diminishes the growth of prostate cancer cell lines, and is thus a potentially viable chemotherapeutic target (28). Additionally, cytotoxic T-lymphocytes specific to AMACR-derived peptides have been shown to exert significant cytotoxic activity against AMACR-expressing prostate cancer cells, suggesting their potential utility as immunotherapy targets in cancer patients (29). The value of AMACR as a prognostic marker may be tissue-type and/or tumor type-dependent. For example, AMACR expression has been found to be a negative prognostic factor in gastric, colorectal and gallbladder carcinomas (30-32), whereas in ovarian, urothelial, renal, and prostatic carcinomas, AMACR expression tends to correlate with negative pathologic factors without being an independent determinant of survival (9,33-34). In contrast, AMACR expression has been found to be a positive prognostic factor in early stage small cell carcinomas of the lung (35). In the present study, we could not demonstrate any relationship between AMACR expression and overall survival in our CCC patients, and AMACR expression was not found to correlate with clinicopathologic factors. However, the definitive resolution of this question will likely require an analysis of a substantially larger dataset than was performed herein.

In summary, we report here that AMACR may have significant utility as a diagnostic adjunct in distinguishing CCC from ESC and EEC. However, its test performance is such that we recommend it be used as part of a panel, rather than as a single marker in the histotyping of endometrial carcinomas.

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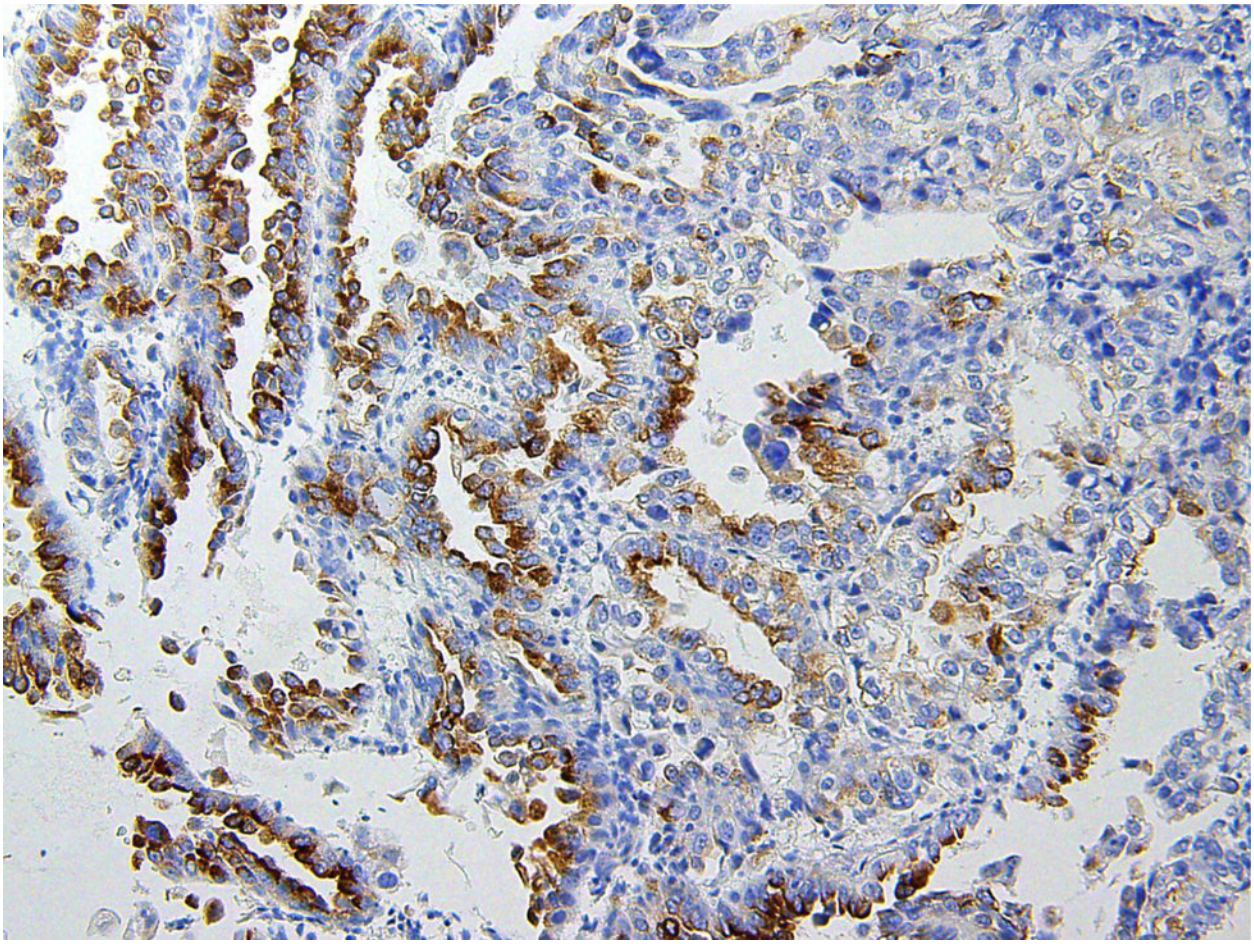


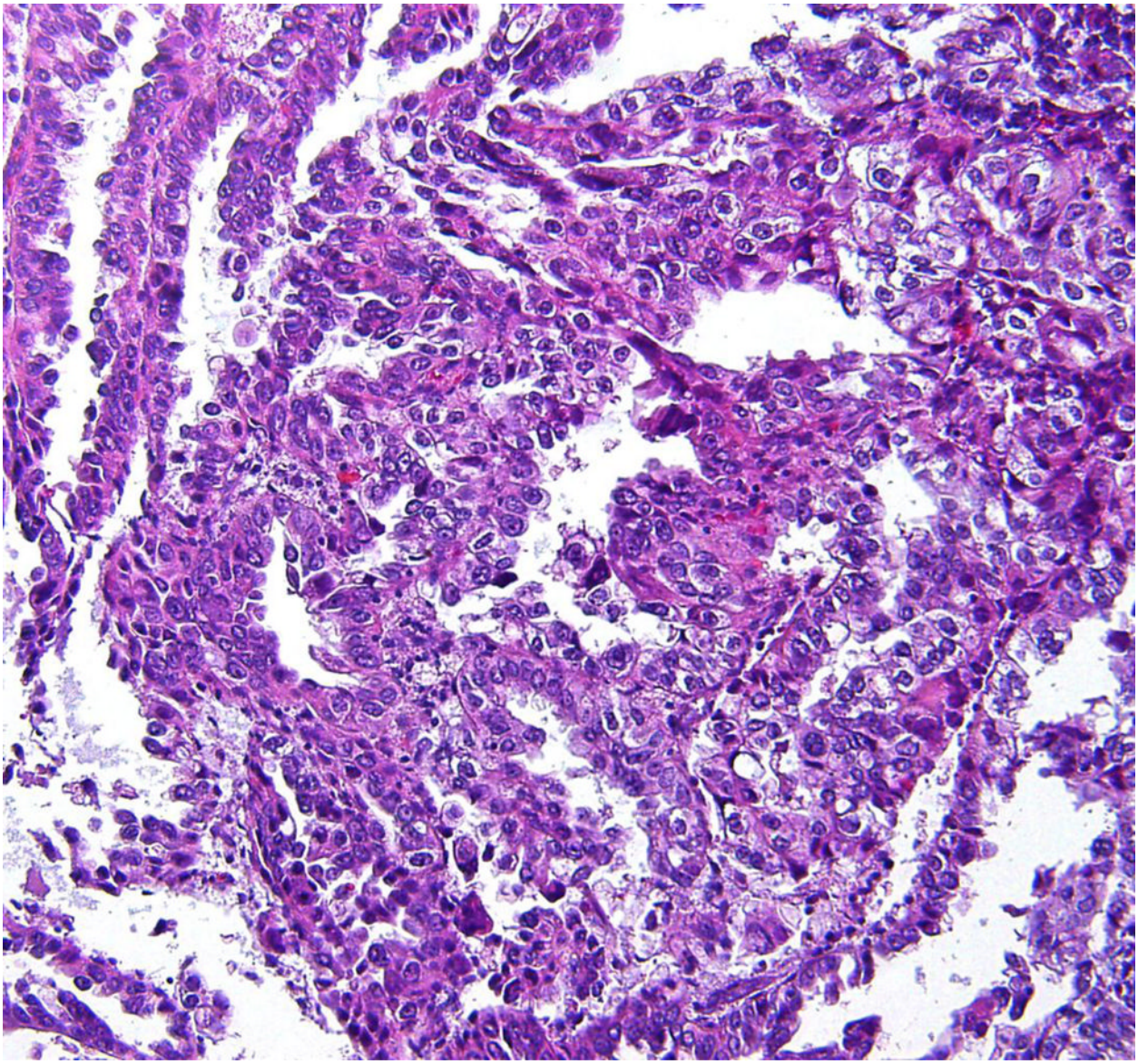
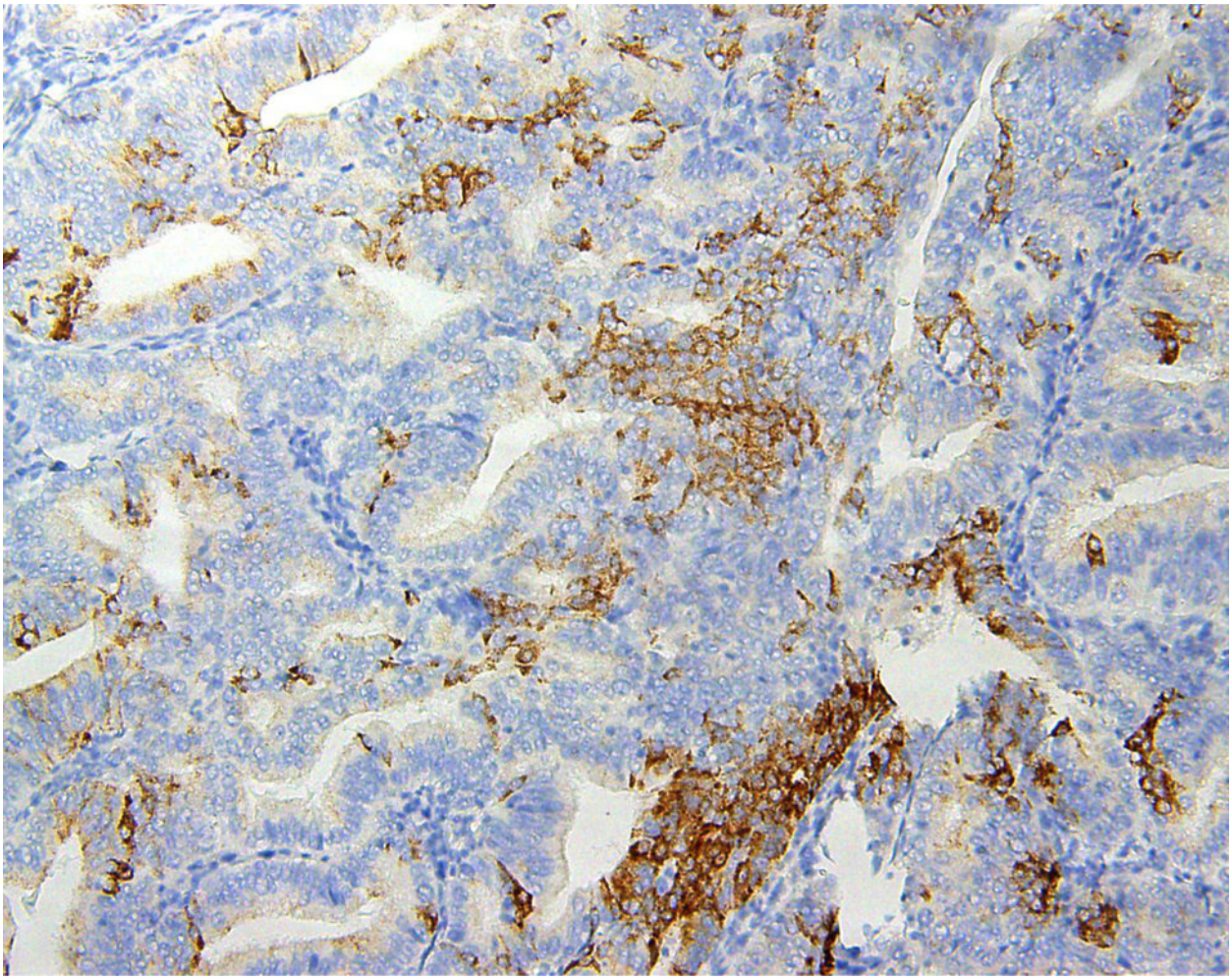


Figure 1.
3+ expression of AMACR in a case of endometrial clear cell carcinoma from the consensus group (figure 1A, AMACR immunohistochemistry, original magnification x200; figure 1B, corresponding hematoxylin and eosin, original magnification x200).



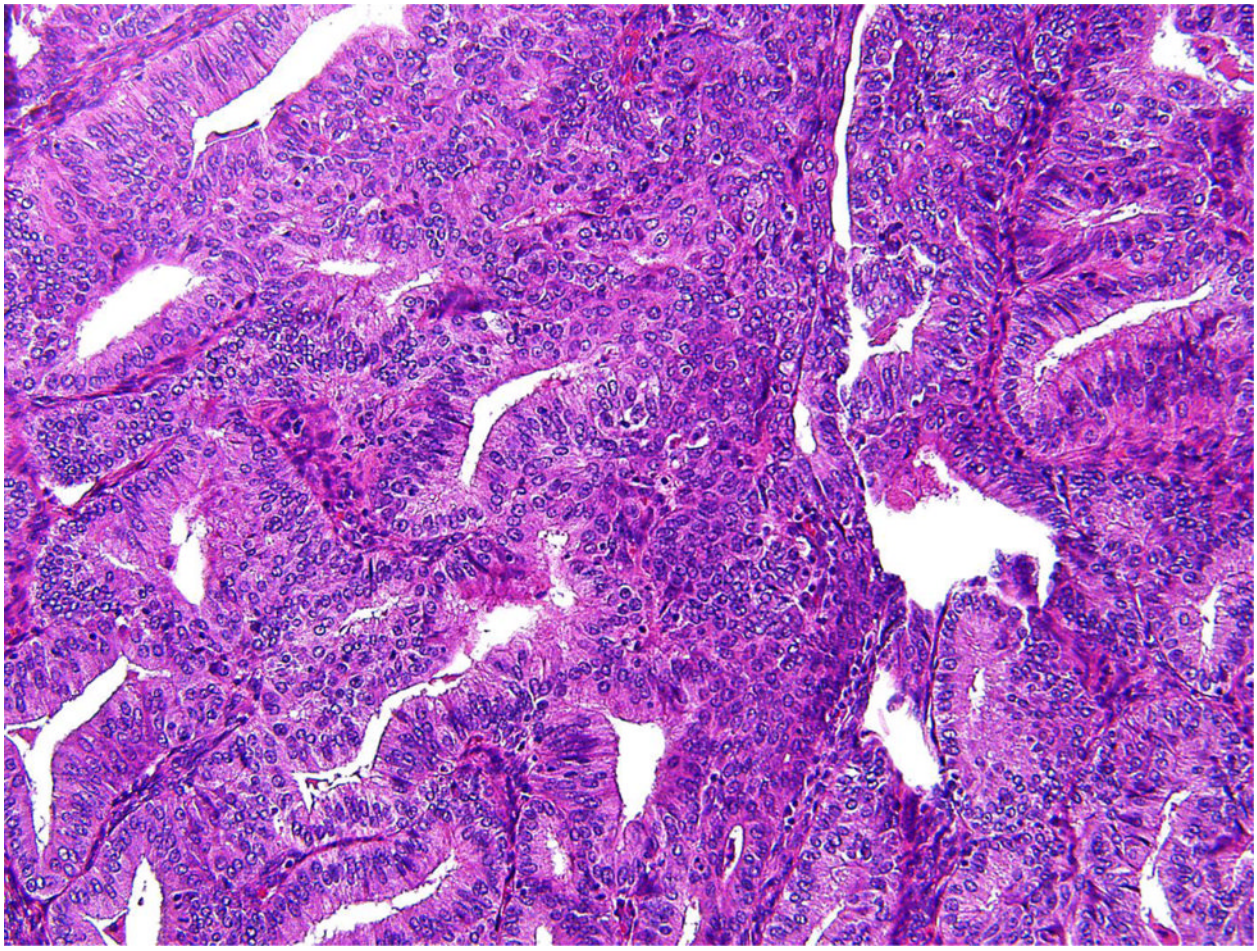
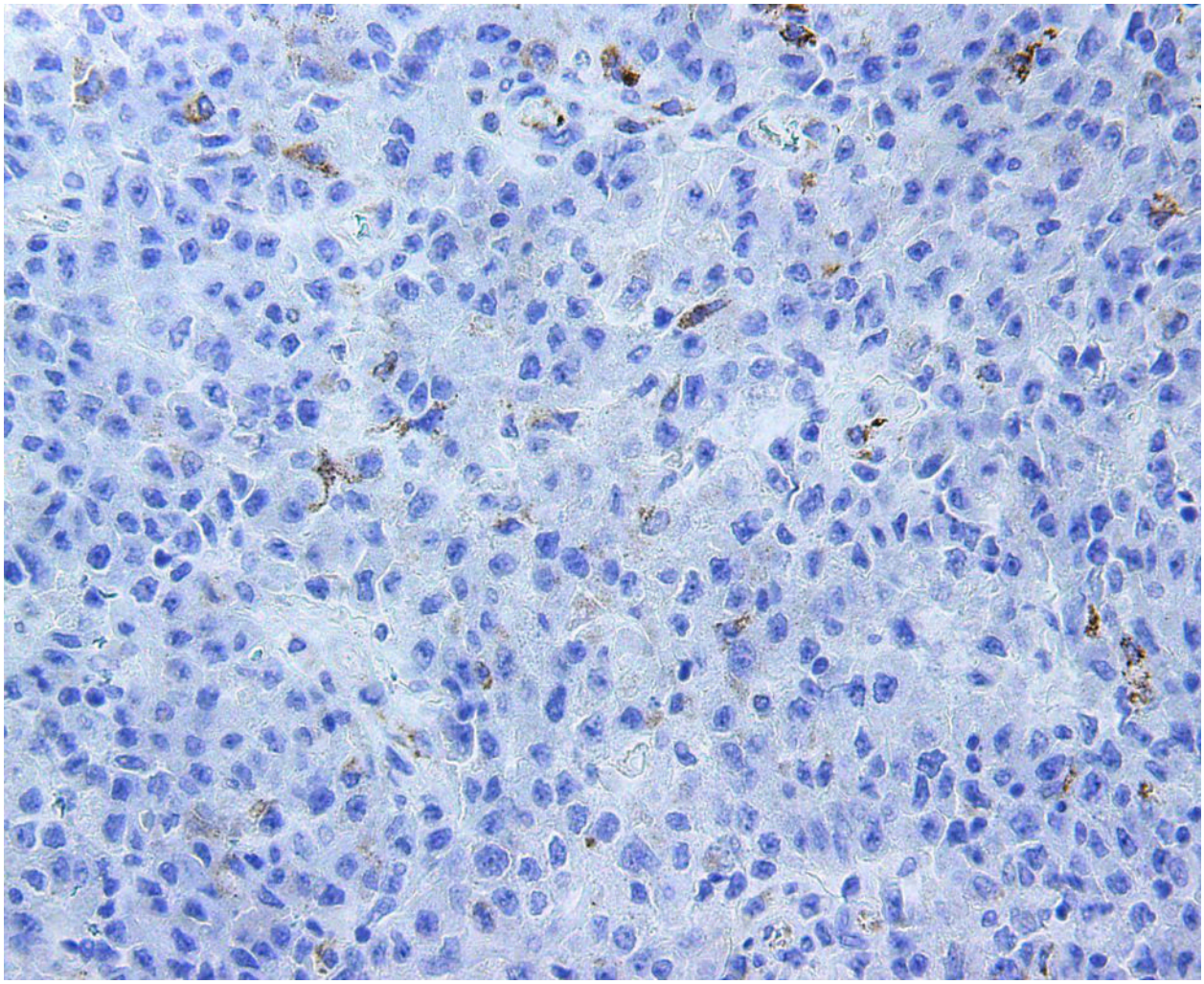


Figure 2.
2+ expression of AMACR in a case of endometrial endometrioid carcinoma (figure 2A, AMACR immunohistochemistry, original magnification x200; figure 2B, corresponding hematoxylin and eosin, original magnification x200).



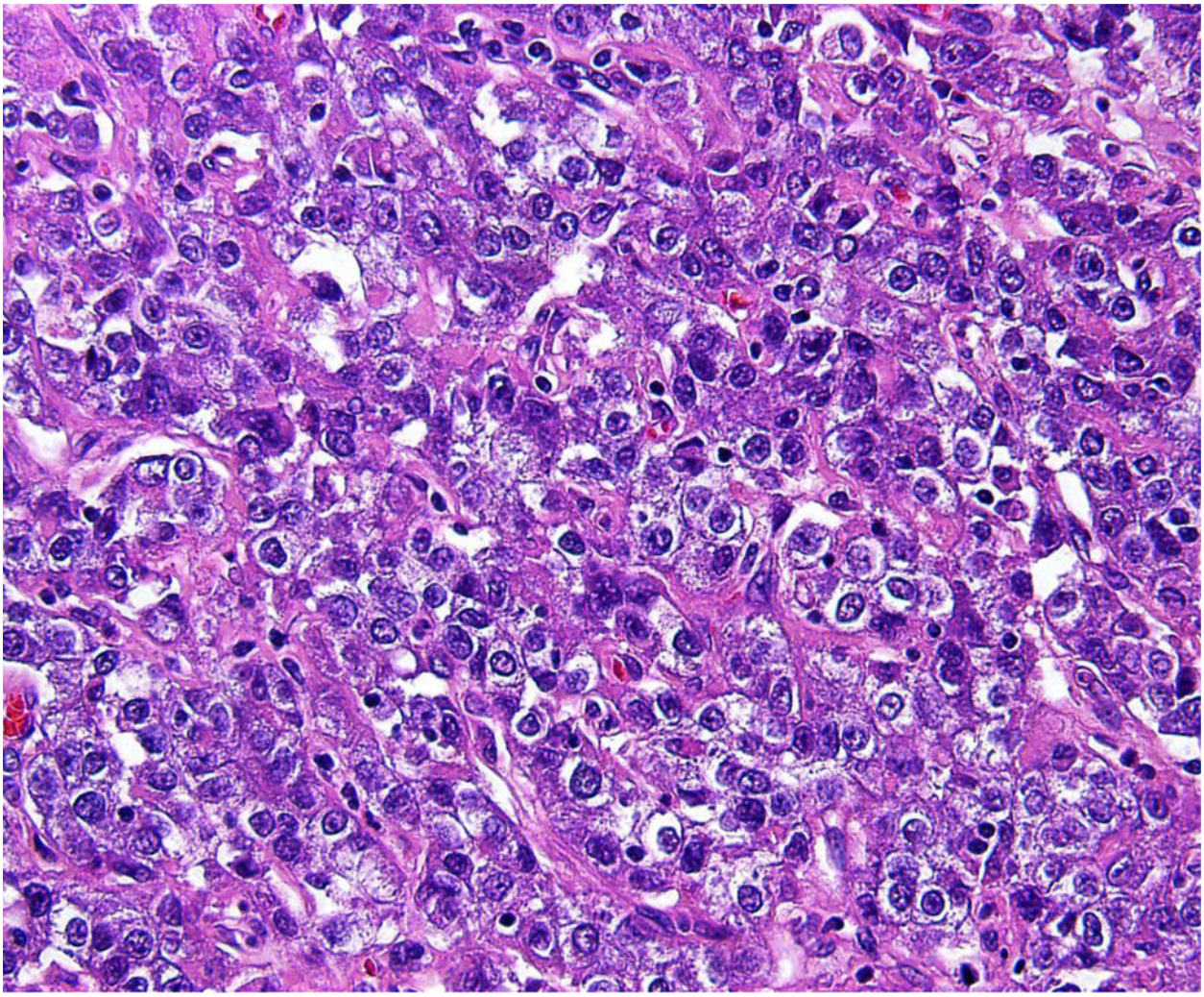


Figure 3.
1+ expression of AMACR in an endometrial carcinoma from the non-consensus group (figure 3A, AMACR immunohistochemistry, original magnification x400; figure 3B, corresponding hematoxylin and eosin, original magnification x400).

Table 1

Distribution of AMACR Immunohistochemical Scores

Tested issues	Number of tested cases	Number of scoreable cases	AMACR immunohistochemical score, number of cases			
			0	1+	2+	3+
Tissue Microarray						
Clear cell carcinoma (Consensus group)	54	49	12	12	3	22
Endometrioid carcinoma	49	49	38	4	4	3
-Grade 3	12	12	11	0	0	1
-Grade 2	19	19	16	2	0	1
-Grade 1	18	18	11	2	4	1
Endometrial serous carcinoma	17	13	11	1	0	1
Non-neoplastic endometrium	25	24	15	5	0	4
-Proliferative	10	9	7	1	0	1
-Secretory	10	10	3	4	0	3
-Atrophic	5	5	5	0	0	0
Whole Tissue Sections**						
Possible clear cell carcinomas (Non-consensus group cases)	17	17	8	4	2	3
Non-neoplastic endometrium	8	8	6	0	2	0
-Arias-Stella reaction	3	3	3	0	0	0
-Clear cell metaplasia	1	1	1	0	0	0
-Secretory endometrium	4	4	2	0	2	0
Other Endometrial carcinomas with clear cells	8	8	7	1	0	0
-Endometrioid carcinoma with secretory changes	4	4	4	0	0	0
-Endometrioid carcinoma with squamous differentiation	2	2	1	1	0	0
-Endometrioid carcinoma with nonspecific clear cells	1	1	1	0	0	0
-Endometrial serous carcinoma with focal clear cells	1	1	1	0	0	0

Scoring scale: 0, 0% tumor epithelial cells positive;

1+, 1-5% positive;

2+, 6-50% positive;

3+,> 50% positive;

** excluding the cases in the TMA verification set

Table 2
Clinicopathologic features of the CCC group

Feature	n (%)
Number of patients	54
Patient age (years)	
Mean	65
Range	50-85
International Federation of Gynecology and Obstetrics (FIGO) stage	
I	21 (39)
II	7 (13)
III	19 (35)
IV	7 (13)
Myometrial Invasion	
Status unknown	1 (2)
Absent	7 (13)
>0% but <50%	19 (35)
>50%	27 (50)
Lymph node status	
Lymph node metastases present	11 (20.4)
Lymph node metastases absent	31 (57.4)
No lymphadenectomy or lymph node status unknown	12 (22.2)
Final status	
Dead of disease	9 (17)
Alive with disease	8 (15)
No evidence of disease	32 (59)
Dead of other causes	1 (2)
Status unknown	4 (7)
Overall survival (months)	
Mean	29.55
Median	20
Range	0.5–104
Relapse-free survival (months)	
Median	15
Range	0.5–104
Mitotic Index (Mitotic figures per 10 High power fields)	
Median	1
Mean	2.4
Range	0-13
0	13 (24)
1	19 (35)

Feature	n (%)
2	6 (11)
3	5 (9)
4	2 (4)
≥5	9 (17)
Necrosis present	26 (48)
Lymphovascular invasion present	28 (52)
Endometrial polyp(s) associated with carcinoma present	13 (24)
Predominant Architectural Pattern	
Papillary	14 (26)
Solid	11 (20)
Tubulocystic	29 (54)
At least 10% of tumor comprised of solid masses	27 (50)
At least 10% of tumor with Individual infiltrating tumor cells	8 (15)
Treatment	
TH/BSO; Adjuvant chemotherapy	12 (22)
Neoadjuvant chemotherapy, TH/BSO, adjuvant chemotherapy	1 (2)
TH/BSO; Adjuvant chemotherapy and radiotherapy	14 (26)
TH/BSO; Adjuvant radiotherapy	13 (24)
TH/BSO without adjuvant treatment	5 (9)
TH/BSO, adjuvant treatment unknown	8 (15)
Neoadjuvant chemotherapy without surgical resection	1 (2)
Relapses	10 (18)
Vagina	2 (4)
Bone	1 (2)
Inguinal region and groin	2 (4)
Kidney	1 (2)
Small bowel	1 (2)
Pleura	1 (2)
Retroperitoneal soft tissue	1 (2)
Supraclavicular lymph node	1 (2)

TH/BSO: total hysterectomy/bilateral salpingo-oophorectomy.