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Original contribution

Addition of IMP3 to L1CAM for discrimination between low- and high-grade endometrial carcinomas: a European Network for Individualised Treatment of Endometrial Cancer collaboration study☆

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Summary Discrimination between low- and high-grade endometrial carcinomas (ECs) is clinically relevant but can be challenging for pathologists, with moderate interobserver agreement. Insulin-like growth factor-II mRNA-binding protein 3 (IMP3) is an oncofoetal protein that is associated with nonendometrioid endometrial carcinomas but has been limited studied in endometrioid carcinomas. The aim of this study is to investigate the diagnostic and prognostic value of IMP3 in the discrimination between low- and high-grade ECs and its added value to L1CAM. IMP3 and L1CAM expression was assessed in tumors from 378 patients treated for EC at 1 of 9 participating European Network for Individualised Treatment of Endometrial Cancer centers. IMP3 was expressed in 24.6% of the tumors. In general, IMP3 was more homogeneously expressed than L1CAM. IMP3 expression was significantly associated with advanced stage, nonendometrioid histology, grade 3 tumors, deep myometrial invasion, lymphovascular space invasion, distant recurrences, overall mortality, and disease-related mortality. Simultaneous absence of IMP3 and L1CAM expression showed the highest accuracy for identifying low-grade carcinomas (area under the curve 0.766), whereas simultaneous expression of IMP3 and L1CAM was strongly associated with high-grade carcinomas (odds ratio 19.7; 95% confidence interval 9.2-42.2). Even within endometrioid carcinomas, this combination remained superior to IMP3 and L1CAM alone (odds ratio 8.6; 95% confidence interval 3.4-21.9). In conclusion, IMP3 has good diagnostic value and together with L1CAM represents the optimal combination of diagnostic markers for discrimination between low- and high-grade ECs compared to IMP3 and L1CAM alone. Because of the homogenous expression of IMP3, this marker might be valuable in preoperative biopsies when compared to the more patchy L1CAM expression.

1. Introduction

Endometrial carcinoma (EC) is the most common gynecological cancer in developed countries and the fourth most common after breast, lung, and colorectal cancer [1]. Patients with localized disease (International Federation of Gynaecology and Obstetrics 2009 stage I and II) have a good prognosis, with a 5-year survival of 95% [1]. However, when there is regional disease spread, the 5-year survival rate is 69%, and in the presence of distant metastases, it is only 17% [1]. Contrary to the decreasing death rates for most cancers, mortality for EC has increased since 2000 [1].

According to the WHO classification, ECs are divided into endometrioid (EECs) and nonendometrioid carcinomas (NEECs), including serous, clear cell carcinomas, carcinosarcomas, and undifferentiated carcinomas [2]. EECs comprise 80% and NEECs 20% of all carcinomas. NEECs are high grade, have a more aggressive course, and subsequently have a worse prognosis [3]. EECs are graded from grade 1 to 3 primarily based on their percentage of solid growth [2]. All NEECs are considered to be grade 3 carcinomas [2]. Grade 3 EECs and NEECs are classically differentiated based on morphological characteristics yet with only moderate inter- and intraobserver agreement [4,5]. A 7-marker immunohistochemical panel was

demonstrated to discriminate grade 3 EEC from serous carcinoma with a 100% concordance rate in a series of 116 cases [4]. L1 cell adhesion molecule (L1CAM) has consistently shown to be a strong prognostic biomarker for identification of patients with poor outcome and is more frequently expressed in NEECs. Although L1CAM is very good in identification of NEECs, only 14%-40% of the grade 3 EECs express L1CAM [6-8]. Because grade 3 EECs have comparable aggressive tumor behavior as NEECs and both need more extensive surgery, the clinical need is mainly to improve identification of high-grade EC [9-14]. Insulin-like growth factor-II mRNA-binding protein 3 (IMP3), also known as L523S or KOC (K-homologous domain-containing protein overexpressed in cancer), is an oncofetal protein that plays a role in tumor growth, migration, and invasion [15]. It is a member of a family of RNA-binding proteins consisting of IMP1, IMP2, and IMP3 [16]. IMP3 is described as a diagnostic and prognostic biomarker in different types of cancer and as a possible therapeutic target [17-20]. In ECs, IMP3 was shown to be more frequently expressed in NEEC, mainly serous carcinomas [21-24]. Although the expression of IMP3 in NEECs might be clinically relevant, expression in EECs has been limited studied [21-25]. Our hypothesis was that IMP3 could contribute to the identification of high-grade EEC in addition to L1CAM and therefore improve discrimination between low- and high-risk ECs. The aim of this study is to investigate the diagnostic and prognostic value of IMP3 in the discrimination between low- and high-grade ECs and its added value to L1CAM.

2. Materials and methods

2.1. Patients

Of 1199 patients from a previously described European Network for Individualised Treatment of Endometrial Cancer

Table 1 Clinicopathological characteristics

	All	IMP3−	IMP3+	P [a]	L1CAM−	L1CAM+	P [a]
No. of patients	378	285 (75%)	93 (25%)		317 (84%)	61 (16%)	
Median age (y)	63 (range 31-88)	63 (range 31-87)	64 (range 37-88)	.109	62 (range 31-87)	69 (range 43-88)	<.001 [c]
Median follow-up [b] (mo)	61 (range 1-205)	62 (range 1-194)	57 (range 6-205)	.221	62 (range 1-205)	54 (range 4-185)	.020 [c]
Treatment							
Lymphadenectomy	236	177 (62%)	59 (63%)	.902	189 (60%)	47 (77%)	.010 [c]
Positive nodes	20	11 (6%)	9 (15%)	.054	12 (6%)	8 (17%)	.035 [c]
Radiotherapy	167	120 (42%)	47 (51%)	.186	136 (43%)	31 (51%)	.318
Chemotherapy	32	16 (6%)	16 (17%)	.001 [c]	21 (7%)	11 (18%)	.007 [c]
FIGO 2009 stage							
Stage I	319	252 (88%)	67 (72%)	<.001 [c]	283 (89%)	36 (59%)	<.001 [c]
Stage II-IV	59	33 (12%)	26 (28%)		34 (11%)	25 (41%)	
Histology							
Endometrioid	346	280 (98%)	66 (71%)	<.001 [c]	310 (98%)	36 (59%)	<.001 [c]
Nonendometrioid	32	5 (2%)	27 (29%)		7 (2%)	25 (41%)	
Grade (only EEC)							
1 or 2	305	258 (92%)	47 (71%)	<.001 [c]	282 (91%)	23 (64%)	<.001 [c]
3	41	22 (8%)	19 (29%)		28 (9%)	13 (36%)	
Myometrial invasion							
<1/2	248	198 (69%)	50 (54%)	.008 [c]	219 (69%)	29 (48%)	.001 [c]
≥1/2	130	87 (31%)	43 (46%)		98 (31%)	32 (53%)	
LVSI							
No	214	168 (59%)	46 (49%)	<.001 [c]	193 (61%)	21 (34%)	<.001 [c]
Yes	53	27 (10%)	26 (28%)		32 (10%)	21 (34%)	
Unknown	111	90 (32%)	21 (23%)		92 (29%)	19 (31%)	
Outcome							
Residual disease	11	6 (2%)	5 (5%)	.148	4 (1%)	7 (12%)	<.001 [c]
Recurrence	43	28 (10%)	15 (17%)	.087	30 (10%)	13 (24%)	.002 [c]
Locoregional	23	19 (7%)	4 (5%)	.615	20 (6%)	3 (6%)	.815
Distant	29	16 (6%)	13 (15%)	.011 [c]	18 (6%)	11 (20%)	.001 [c]
Deceased	51	32 (11%)	19 (20%)	.035 [c]	32 (10%)	19 (31%)	<.001 [c]
Endometrial cancer	30	15 (5%)	15 (16%)	.002 [c]	16 (5%)	14 (23%)	<.001 [c]

Abbreviations: ;FIGO, International Federation of Gynaecology and Obstetrics; ;LVSI, lymphovascular space invasion.

[a] P for χ^2 test for categorical variables. For nominal variables Mann-Whitney U test was performed.

[b] Median follow-up including deceased patients.

[c] Statistically significant.

collaboration study cohort, 400 were randomly selected [6]. The randomly selected patients were not statistically different from the original cases for all variables shown in Table 1. Of these 400 selected patients, 19 patients were excluded because no more blank slides were available, and 3 patients were excluded after immunohistochemical staining due to lack of EC tissue. In total, 378 patients were included in the present study. The cohort consists of patients treated for an EC at one of the collaborating European Network for Individualised Treatment of Endometrial Cancer centers. Only patients with tumors diagnosed by a dedicated gynecological pathologist with complete data on treatment and pathology, including follow-up data of at least 36 months, were selected. Clinical and pathological data were recorded from the patient files into a database.

2.2. Tissue and staining

Blank 4-μm–thick sections were cut from formalin-fixed, paraffin-embedded tissue blocks and were sent to the Radboud university medical center. After antigen retrieval, achieved with the Thermo Scientific PT Module (Waltham, MA, USA) in EDTA pH 9 for 10 minutes and endogenous peroxidase blocking, slides were incubated with IMP3 antibody (Clone 69.1, Dako, Glostrup, Denmark, dilution 1:50). They were subsequently incubated with PowerVision+ Poly-HRP and visualized with PowerVision DAB substrate solution (Leica Biosystems, Buffalo Grove). Finally, the slides were counterstained with hematoxylin, dehydrated, and mounted. L1CAM staining was performed as described previously [6].

2.3. Scoring

Slides stained for IMP3 were scored semiquantitatively. The final score was the product of the staining intensity and staining area scores of the cytoplasmic staining. Staining intensity was graded from 0 (no staining) to 3 (strong staining) (Fig. 1). The area was scored as 0 (no tumor cells positive), 1 (<10% of the tumor cells positive), 2 (10%-50% of the tumor cells positive), and 3 (>50% of the tumor cells positive). Scoring was independently performed by 2 investigators (N. C. M. V. and A. E.). In case of disagreement, the case was discussed, and a consensus score was determined. For IMP3, a staining index of $\geq$4 was considered as positive [25]. L1CAM membranous expression was scored as previously described, and L1CAM was considered to be positive in case of >10% stained tumor cells [6].

2.4. Statistical analysis

Clinicopathological differences between IMP3-negative and -positive tumors were calculated with χ^2 and Fisher exact tests for categorical and the Mann-Whitney U test for continuous variables. Interobserver variability for IMP3 score was calculated using Cohen κ.

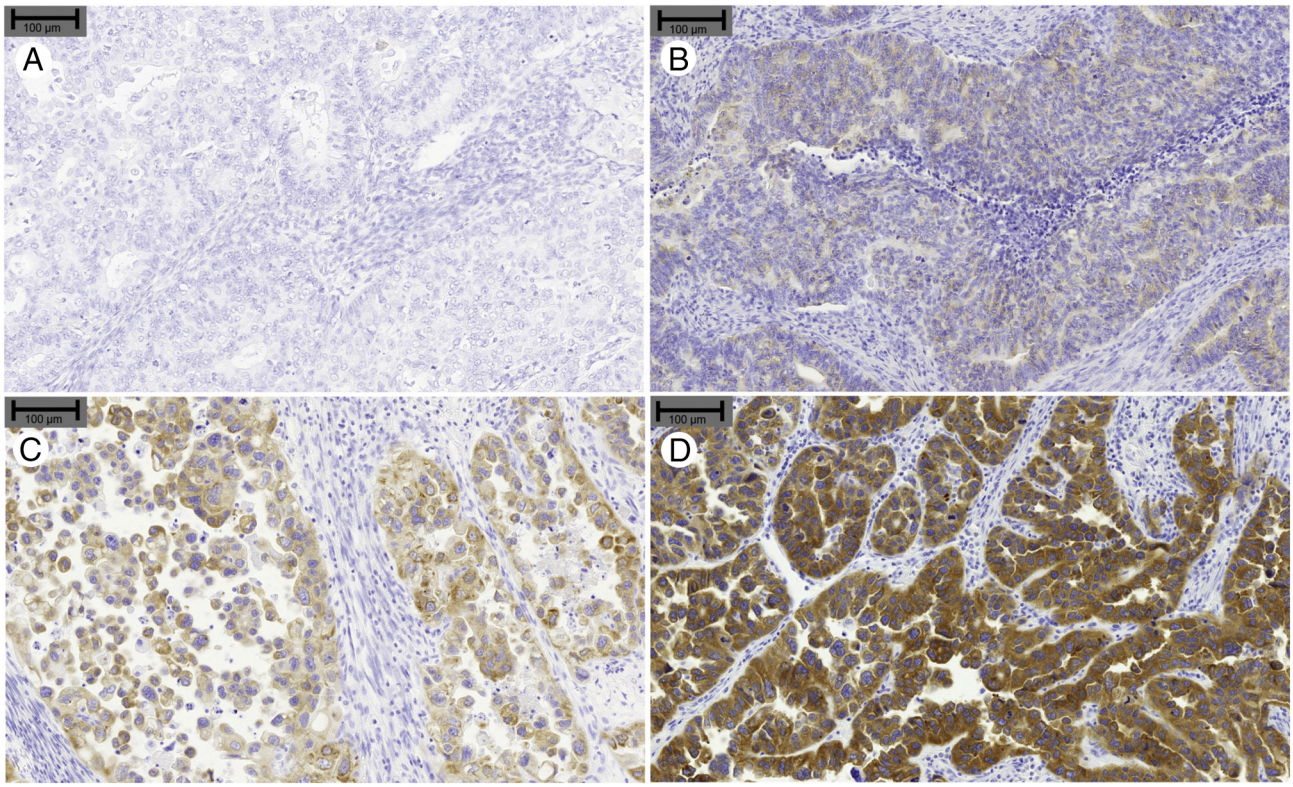

Fig. 1 IMP3 staining intensities. A, Negative; (B) weak; (C) moderate; (D) strong.

To investigate the diagnostic value of IMP3 and its added value to L1CAM, a receiver operating characteristic (ROC) curve was constructed for the discrimination between low- and high-grade ECs based on IMP3 and L1CAM expression. *Low-grade ECs* were defined as histological grade 1 or grade 2 differentiated endometrioid carcinomas, and *high-grade ECs* were defined as histological grade 3 differentiated endometrioid carcinomas and all nonendometrioid carcinomas. The sensitivity, specificity, positive predictive value, negative predictive value, and the area under the ROC curve (ROC AUC) were calculated. Binary logistic regression was used to calculate odds ratios (ORs) and 95% confidence interval (CI) for discrimination between low- and high-grade ECs.

Subsequently, we performed the same analyses within the subgroup of patients with endometrioid ECs and for the discrimination between endometrioid and nonendometrioid carcinomas.

To analyze the prognostic value, hazard ratios (HRs) and 95% CI for 5-year overall and recurrence-free survival in relation to IMP3 and L1CAM expression were calculated using Cox regression analysis.

A $P < .05$ was considered to be significant for statistical differences. SPSS version 21 (SPSS IBM, Armonk, NY) statistical software was used to perform the statistical analyses.

2.5. Ethical approval

The study was approved by the institutional review board of all participating centers.

3. Results

Clinicopathological characteristics of the 378 included patients are shown in Table 1, as well as characteristics of patients separated according to IMP3 and L1CAM expression. Of the 378 included patients, 346 had an EEC and 32 an NEEC. Of the patients with an NEEC, the primary nonendometrioid component was serous in 19 patients, clear cell in 8, carcinosarcoma in 4, and undifferentiated in 1 patient.

3.1. Expression of IMP3 and L1CAM

There was a substantial agreement in IMP3 scoring between the 2 observers ($\kappa = 0.78$). IMP3 was expressed in 24.6% (n = 93) of the tumors. IMP3 expression was significantly associated with advanced stage, nonendometrioid type, grade 3 tumors, deep myometrial invasion, lymphovascular space invasion, distant recurrences, overall mortality, and disease-related mortality (Table 1).

IMP3 expression was observed in 15% of the grade 1 and 2 EECs, 46% of the grade 3 EECs, and 84% of the NEECs (Table 2). Within the different subgroups of NEECs, 84% of the serous carcinomas, 75% of clear cell carcinomas, and all carcinosarcomas and undifferentiated carcinomas were positive for IMP3. L1CAM expression was present in 8% of the grade 1 and 2 EECs, 32% of the grade 3 EECs, and 78% of the NEECs.

Table 2 IMP3 and L1CAM expression in different subgroups

IHC marker	EEC grade 1-2	EEC grade 3	NEEC	Total
IMP3+/L1CAM+	11 (26%)	10 (24%)	21 (50%)	42
IMP3+/L1CAM−	36 (71%)	9 (18%)	6 (12%)	51
IMP3−/L1CAM+	12 (63%)	3 (16%)	4 (21%)	19
IMP3−/L1CAM−	246 (93%)	19 (7%)	1 (0.4%)	266

Most IMP3-negative tumors were also negative for L1CAM (93%) (Table 2). Of the IMP3-positive tumors, 45% showed L1CAM expression, whereas 55% were L1CAM negative. These IMP3-positive, L1CAM-negative tumors were mainly endometrioid tumors (88%) with low stage (88%) and low grade (71%). Examples of positive and negative tumors are illustrated in Fig. 2. In general, IMP3 showed a more homogeneous expression pattern compared to L1CAM, which more often showed a patchy expression (Fig. 2J-L).

3.2. Diagnostic value

High-grade ECs more frequently express IMP3 (63%) than L1CAM (52%). Absence of both IMP3 and L1CAM expression was associated with low-grade EC (OR 11.0; 95% CI 6.1-19.9) and showed the highest AUC for discriminating between low- and high-grade ECs (AUC 0.766) (Table 3). In case a tumor was negative for both IMP3 and L1CAM, there was a 93% chance the EC was low grade. A combination with 1 positive marker and 1 negative marker had less diagnostic value, both within a combination of EECs and NEECs and within the subgroup of EECs (Table 3). A combination of both IMP3 and L1CAM expression was strongly associated with high-grade EEC and NEEC (OR 19.7; 95% CI 9.2-42.2). Results for the different combinations of IMP3 and L1CAM within the subgroup of EECs were comparable. Within the subgroup EECs, combined IMP3 and L1CAM expression was strongly associated with high-grade EC (OR 8.6; 95% CI 3.4-21.9).

For differentiating between EECs and NEECs, both a combination of IMP3 and L1CAM and use of IMP3 and L1CAM individually were accurate markers (Supplementary Table S1). A combination of negative IMP3 and negative L1CAM expression showed both a very high positive predictive value for diagnosing EEC (100%) and a high negative predictive value (72%), and was better than L1CAM alone (Supplementary Table S1). Results for discrimination between EECs and NEECs within high-grade EC were comparable.

3.3. Prognostic value

Overall, patients with tumors expressing IMP3 showed a reduced 5-year overall survival (HR 2.0; 95% CI 1.1-3.8) without a significant difference in recurrence-free survival

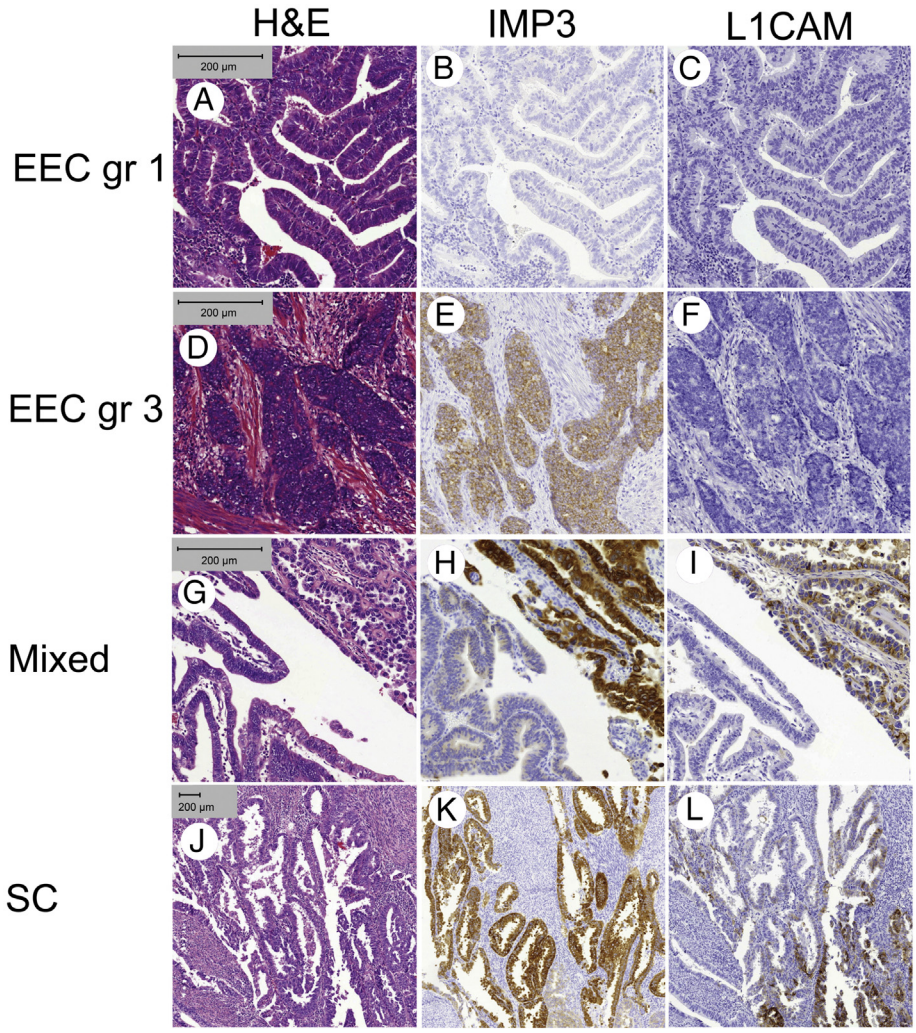

Fig. 2 IMP3 and L1CAM expression, representative cases. A-C, Grade 1 EEC with negative IMP3 (B) and L1CAM (C) staining; (D-F), grade 3 EEC with positive IMP3 (E) and negative L1CAM (F) staining; (G-I) mixed carcinoma with EEC component negative for both IMP3 and L1CAM (H and I) and clear cell component positive for both IMP3 and L1CAM (H and I); (J-L) serous carcinoma (SC) with homogeneous expression of IMP3 (K) and patchy expression of L1CAM (L).

(HR 1.9; 95% CI 0.97-3.6). Tumors expressing L1CAM showed both a reduced overall survival (HR 4.7; 9% CI 2.5-8.7) and recurrence-free survival (HR 2.8; 95% CI 1.4-5.6).

However, within the subgroup of patients with stage I EECs, neither IMP3 (HR 1.2; 95% CI 0.5-3.4) nor L1CAM (HR 1.4; 95% CI 0.4-4.9) showed a significant difference in recurrence-free and overall survival (HR 1.1; 95% CI 0.3-3.9 and HR 2.4; 95% CI 0.7-8.4, respectively). In addition, within the subgroup of patients with low-grade EECs, neither IMP3 (HR 0.9; 95% CI 0.3-3.1) nor L1CAM (HR 0.6; 95% CI 0.1-4.6) showed a significant difference in recurrence-free and overall survival (HR 0.8; 95% CI 0.2-3.5 and HR 1.8; 95% CI 0.4-7.7, respectively).

Table 3 Logistic regression analysis of the prediction of low- and high-grade endometrial carcinoma in all included patients

IHC marker	OR	95% CI	Sensitivity	Specificity	PPV	NPV	ROC AUC	Positive level
IMP3+	9.4 [a]	5.3-16.5	63%	85%	50%	91%	0.738	Grade 3
L1CAM+	13.3 [a]	7.1-24.9	52%	93%	62%	89%	0.723	Grade 3
IMP3+/L1CAM+	19.7 [a]	9.2-42.2	43%	96%	74%	88%	0.694	Grade 3
IMP3+/L1CAM–	1.9	0.99-3.8	21%	88%	29%	82%	0.544	Grade 3
IMP3–/L1CAM+	2.6	0.98-6.8	10%	96%	37%	82%	0.528	Grade 3
IMP3–/L1CAM–	11.0 [a]	6.1-19.9	81%	73%	93%	47%	0.766	Grade 1-2

Abbreviations: NPV, Negative predictive value; PPV, Positive predictive value.
[a] Statistically significant.

4. Discussion

In this large multicenter study, we showed that IMP3 has additional value as diagnostic biomarker in ECs. Combination of IMP3 and L1CAM expression was demonstrated to be superior to IMP3 and L1CAM alone for the discrimination between low- and high-grade ECs.

IMP3 is a novel marker that was reported to be more frequently expressed in serous compared to EECs [21-24]. In 3 studies that evaluated 118, 122, and 311 EECs, respectively, IMP3 expression was more often found in high-grade EECs compared (20%-39%) to low-grade EECs (3%-9%), which is in line with the results of our study where we found expression in 46% and 15% of the high- and low-grade EECs, respectively [21,22,25]. Both Mhawech-Fauceglia et al (2013) and Li et al used a combination of intensity and percentage of positive cells [22,25]. However, 1 study did not find a relation between IMP3 expression and grade in EECs [24]. This conflicting result might be explained by the limited amount of patients with EEC in the latter study (n = 57) and the different scoring system used (≥5% staining scored as positive) [24].

During the last years, more and more diagnostic and prognostic markers, such as estrogen receptor, progesterone receptor, and p53 have been studied in ECs [26]. Recently, L1CAM has established its role as a prognostic biomarker in ECs [6,27-29]. Dellinger et al have studied L1CAM gene expression in The Cancer Genome Atlas RNA-seq dataset and have shown that L1CAM gene expression is an independent predictor of poor survival in EC patients [30]. IMP3 is a relatively new marker, and it has not been extensively studied in EECs. Although the pathophysiological mechanisms of L1CAM and IMP3 are quite different, both show increased expression in NEECs and are associated with aggressive tumor characteristics [6,15,16,27-29,31]. Previous studies have analyzed the relationship between L1CAM, p53, estrogen receptor, and progesterone receptor but focused on prognostic rather than diagnostic capacity [7,32]. To our knowledge, this is the first study investigating the added value of IMP3 to L1CAM as diagnostic biomarker. We have shown that combining these 2 biomarkers significantly improves the discrimination between low- and high-grade ECs in the present study cohort compared to both markers individually.

NEECs and high-grade EECs require more extensive surgical treatment than low-grade EECs, which means that distinguishing low- and high-grade ECs is highly clinically relevant. Discrimination between low- and high-grade ECs based on morphology alone can be challenging for pathologists, with only a moderate interobserver agreement in EECs [33-35]. Addition of immunohistochemical stainings might improve this agreement. Because L1CAM is expressed in only 14%-40% of the grade 3 EECs, this is a limitation in its use for identification of all patients with high-grade carcinomas [6-8]. Even with some overlap in IMP3 and L1CAM expression, we found more IMP3 expression in both grade 3 EECs and NEECs compared to L1CAM. The present study has shown that the combination of IMP3 and L1CAM expression is most optimal to discriminate between low- and high-grade ECs. IMP3-negative, L1CAM-negative tumors were 11 times more likely to be low grade than ECs expressing 1 or both of these markers. These findings remained the same when NEECs were excluded. The high positive predictive value of this combination might be useful in selecting patients that do not require extensive surgery or adjuvant therapy.

Although a combination of IMP3 and L1CAM is optimal to discriminate between low- and high-grade ECs, this combination showed comparable results with L1CAM for discrimination between EECs and NEECs. However, because patients with high-grade EECs NEECs have comparable outcome, discrimination between low- and high-grade ECs is more clinically relevant than the discrimination between EECs and NEECs [9-12].

Current results are based on the hysterectomy specimen, whereas the surgical treatment is determined on the preoperative histological diagnosis. Overall, there is only 67% agreement concerning tumor grade between preoperative endometrial sampling and final diagnosis [36]. Addition of immunohistochemistry might help differentiating between low- and high-risk patients preoperatively. The immunohistochemical stains in the current study were performed on whole slide, whereas most previous studies on the prognostic value of IMP3 have used tissue microarray [21,23,25]. Based on preliminary data of own research, IMP3 showed a more homogeneous expression than L1CAM. The more homogeneous expression might be important in case of staining on preoperative biopsies. Therefore, IMP3 might be valuable in identification of high-risk patients on preoperative histology.

The strength of this study is the large number of included patients of different grades and types, and the median follow-up of 61 months (range 1-205 months). This long follow-up minimized the chance of missing recurrences and deaths.

A limitation of this study is the retrospective design which could cause a selection bias. We did not find additional prognostic value of IMP3. In contrast to the previous study, we did not find a prognostic value of L1CAM in patients with stage I EECs in this randomly selected cohort of 400 patients out of 1199 from the original study cohort [6]. Therefore, the lack of prognostic value of IMP3 in this study might be because of selection bias or the limited number of patients.

The histology was not revised centrally. However, all participating pathologists were dedicated gynecological pathologists. Whether this is a limitation or strength could be debated. By using the local histological diagnoses, the results of this study are applicable to daily practice in hospitals which employ dedicated pathologists.

5. Conclusions

In conclusion, IMP3 has good diagnostic value and together with L1CAM represents the optimal combination of

diagnostic markers for discrimination between low- and high-grade ECs compared to IMP3 and L1CAM alone, regardless of histological type. Because of the homogenous expression of IMP3, this marker might be valuable in preoperative biopsies when compared to the more patchy L1CAM expression.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2019.04.014.

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